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(54) Title: LIQUID DISTRIBUTION SYSTEM

(57) Abstract

The present invention provides a liquid distribution system, which is useful in a number of contexts, including in accomplishing various synthetic, diagnostic and drug screening reactions. The distribution system can comprise an alpha reservoir (200A) and a beta reservoir (200B), a first set of parallel and adjacent first and second feeder channels (211A-211B) and a second set of parallel and adjacent third and fourth feeder channels (211C-211D) which are offset from the first and second feed channels (211A-211B), wherein (a) the first (211A) and third (211C) feeder channels are connected to the alpha reservoir (200A) via a first connector channel (211A) that is situated above or below the second (211B) and fourth feeder channels (211D) and are independent of the beta reservoir and (b) the second (211B) and fourth feeder channels (211D) are connected to the beta reservoir via a second connector channel (211B) that it situated above or below the first and third feeder channels (211A and 211C) and are independent of the alpha reservoir (200A). The distribution system is preferably a microscale distribution system. Various particular mechanisms for controlling flow into a liquid distribution system are described.

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LIQUID DISTRIBUTION SYSTEM

This application relates to a method and system for manipulating fluids, which is useful in a number of contexts, including in accomplishing various synthetic, diagnostic and drug screening reactions.

5   More particularly, this invention relates to a system and method, which incorporates a layered array, for distributing reagent liquids while inhibiting the contamination or cross-contamination of these liquids.

Recently, a number of academic articles have focused on the problems associated with conducting chemical reactions on a micro-scale.

10 This literature has discussed the possibility of managing such reactions on wafer-sized solid supports that have been etched to create microchannels. Reactor systems of this scale could allow multiple diagnostic or drug screening assays to be conducted in a transportable device that uses small amounts of reagents, thus reducing supply and disposal costs.

15   One mechanism for developing new drugs not provided for by nature has been dubbed "rational" drug design. This process looks at the structures of biological macromolecules as determined by crystallography and at the structures of pharmacological agents known to interact with these macromolecules. With the use of computer workstations, it was hoped that

20 new pharmacological agents could be designed that had appropriately positioned functionalities for strongly interacting with the macromolecule. One difficulty with this approach is that growing crystals appropriate for crystallographic structural determinations is a tedious, empirical science. In many cases, it is unclear if appropriate crystals can be grown (for instance,

25 for the glycoprotein hormones such a chorionic gonadotropin or other glycoproteins). Another difficulty is that chemistry does not provide the malleable construction tools evoked by the phrase "design"; instead, chemical building blocks provide only a limited number of bond angles and lengths. For example, the structural routes by which a chlorine group might be positioned

30 in particular part of a drug-binding pocket in the macromolecule may be many, while the advantages or disadvantages of the ancillary structures needed to position this group are hard to "rationally" evaluate.

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Combinatorial chemistry seeks to create its own "evolutionary" process that selects for compounds with the desired pharmacological activity. The key to making the process evolutionary is to generate large families of "mutants", in this case families of compounds with

5 some chemical relatedness but with clear differences. The concepts of rational design may be taken advantage of in selecting the families of compounds to be explored by the combinatorial method.

Combinatorial chemistry seeks to generate new leads to classes of compounds that have potential pharmacological activity.

10 Traditionally, such leads have been found by screening various plant or animal extracts for pharmacological activity. Such extracts are tedious to obtain, may have very small concentrations of potentially useful compounds, and at best only contain compounds selected by evolutionary pressures that may have nothing to do with the disease that is sought to be treated. After an

15 extract has been identified, the process provides little information as to the identity of the active ingredient.

Combinatorial chemistry seeks to create the large, diverse family of compounds by permutation of a relatively limited set of building block chemicals. Preferably, the combinatorial method will create identifiable

20 pools containing one or more synthetic compounds. These pools need not be identifiable by the chemical structure of the component compounds, but should be identifiable by the chemical protocol that created the compounds. These pools are then screened in an assay that is believed to correlate with a pharmacological activity. Those pools that produce promising results are

25 examined further to identify the component compounds and to identify which of the component compounds are responsible for the results.

The follow-up protocol used to identify the active compounds in a combinatorial pool can also involve a combinatorial method. For instance, the promising pool could result from the reaction, first, of a mixture of

30 compounds A, B and C, which compounds do not react with one another, with compounds D, E and F, which compounds do not react with one another but do react with compounds A, B or C. Second, the resulting compounds

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are reacted with compounds G, H and I. To narrow the possible identity of

the active compounds in the pool, the A-D, A-E, A-F, B-D, B-E, B-F, C-D, C-E

and C-F products can be separately created by combinatorial chemistry and

separately reacted with a the mixture of G, H and I. After this step, the

5   sub-pool that is active in the screening assay generally will contain a more

limited family of compounds.

Once promising molecules are identified by combinatorial

chemistry, the identified molecules provide information that aides in the

design of further combinatorial experiments. The full array of promising

10   compounds identified by combinatorial chemistry can provide valuable

information to guide traditional pharmaceutical chemistry efforts.

A popular tool in the emerging field of combinatorial

chemistry is to attach the first chemical building blocks to solid support,

typically a glass or polymeric support, such as the supports used in the well

15   known Merrifield method for synthesizing polypeptides. This attachment

provides a mechanism for quickly isolating product by simply washing away

reactants and related impurities and decoupling the product from the support.

In some cases, the support-coupled product can be assayed for

pharmacological activity.

20   Miniaturization is usefully employed in combinatorial

chemistry since: (i) workers generally seek compounds that are

pharmacologically active in small concentrations; (ii) in creating a vast

"evolutionary" assortment of candidate molecules it is preferable to have the

numerous reactions well documented and preferably under the direction of a

25   limited number of workers to establish reproducibility of technique; (iii) it is

expensive to create a vast, traditionally-scaled synthetic chemistry complex

for creating a sufficiently diverse family of candidate compounds; and (iv)

substantial concerns are raised by the prospect of conducting assays of the

products of combinatorial chemistry at more standard reaction scales.

30   Miniaturization allows for the economic use of robotic control, thereby

furthering reproducibility.

The wafer-sized devices described above can be ideal for

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combinatorial chemistry, allowing for numerous synthetic chemistry reactions
to be conducted substantially under computer control using only small
quantities of reagents. However, the academic literature advocating such
micro-scale devices has not adequately addressed fundamental issues in

5   conducting combinatorial chemistry at this scale: for instance, how does one
manage to shuttle reagents through a complex microscale device and
accomplish this without significant cross-contamination while allowing a
complex assortment of different syntheses to occur in a large number of
microscale reaction vessels (e.g., 100 to 10,000) in the device? The present

10   invention provides a microscale device that solves these issues.
SUMMARY OF THE INVENTION

The present invention provides a liquid distribution system,
which is useful in a number of contexts, including accomplishing various
synthetic, diagnostic and drug screening reactions. The distribution system

15   can comprise an alpha reservoir and a beta reservoir, a first set of parallel and
adjacent first and second feeder channels and a second set of parallel and
adjacent third and fourth feeder channels which are offset from the first and
second feeder channels, wherein (a) the first and third feeder channels are
connected to the alpha reservoir via a first connector channel that is situated

20   above or below the second and fourth feeder channels and are independent of
the beta reservoir and (b) the second and fourth feeder channels are
connected to the beta reservoir via a second connector channel that is
situated above or below the first and third feeder channels and are
independent of the alpha reservoir. The distribution system is preferably a

25   microscale distribution system.
BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a system of channels for addressing any of
one hundred reaction cells with any of four fluids.

Figure 2 displays a distribution plate according to the

30   invention.

Figure 3 displays an expanded view of a portion of the
distribution plate of Figure 1.

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Figure 4A shows a capillary barrier between a first distribution channel and a buffer channel.

Figure 4B shows a capillary barrier between a first distribution channel and a buffer channel and another capillary barrier between a buffer channel and a vertical channel.

Figure 5 shows in three dimensions various cut-away views of the feedthrough plate, distribution plate and reaction cell plate of a distribution system of the invention.

Figure 6A shows a cross-section along an EW axis through a distribution channel.

Figure 6B shows a cross-section along an EW axis through a first reservoir.

Figure 7 is a top view of a part of a distribution plate.

Figure 8 shows a voltage pulse pattern used to power an electrode-based pump useful in the liquid distribution system of the invention.

Figure 9 schematically illustrates a liquid distribution system of the preferential flow liquid distribution system.

Figures 10A and 10B show cross-sectional views of a liquid distribution system of the preferential flow liquid distribution system.

Figure 11 shows a schematic top view of a liquid distribution system of the preferential flow liquid distribution system.

Figure 12 shows a top view of a liquid distribution system of the expansion valve liquid distribution system.

Figures 13A - 13C show cross-sectional views of various embodiments of the expansion valve liquid distribution system

Figure 14 shows a top view of a portion of an electrode-based liquid distribution system.

Figures 15A and 15B illustrate reaction cell designs.

Figures 16A - 16D show various capillary barrier designs.

Figure 17 shows a device for conducting field assisted bonding of plates.

Figures 18A and 18B show a channel device having

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electrode-based pumps.

Figure 19 shows a liquid distribution system design pursuant to the hydrologic liquid distribution system.

Figure 20 shows a reaction cell having a heater and a

5 thermocouple.

Figures 21A and 21B show a valve design.

DEFINITIONS

The following terms shall have the meaning set forth below:

- addressable

a reaction cell or channel is "addressable" by a reservoir or another channel if liquid from the reservoir or other channel can be directed to the reaction cell or channel.

10 • adjacent

"adjacent" as used in these situations: (i) a first structure in one of the plates is adjacent to a second structure in the same or another plate if the vertical projection of the first structure onto the plate of the second structure superimposes the first structure on the second or places it within about 250 μm of the second; and (ii) groupings of two or more channels are adjacent to one another if each channel is in substantially the same horizontal plane, and all but the outside two channels in the grouping are adjacent (in the sense defined in (i) above) to two neighbor channels in the grouping. Preferably, under item (i), a first structure is adjacent to a second structure if the vertical projection of the first structure onto the plate of the second structure superimposes the first structure on the second or places it within about 150 μm of the second.

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• capillary dimensions	dimensions that favor capillary flow of a liquid. Typically, channels of capillary dimensions are no wider than about 1.5 mm. Preferably channels are no wider than about 500 μm, yet more preferably no wider than about 250 μm, still more preferably no wider than about 150μm.
• capillary barrier	a barrier to fluid flow in a channel comprising an opening of the channel into a larger space designed to favor the formation, by liquid in the channel, of an energy minimizing liquid surface such as a meniscus at the opening. Preferably, capillary barriers include a dam that raises the vertical height of the channel immediately before the opening into the larger space.
• connected	the channels, reservoirs and reaction cells of the invention are "connected" if there is a route allowing fluid between them, which route does not involve using a reaction cell as part of the link.
• continuous flow channel	a channel having an overflow outlet allowing for fluid to continuously flow through the channel.
• directly connected	reservoirs and horizontal channels are "directly connected" if they are connected and either (1) no other channel is interposed between them or (2) only a single vertical channel is interposed between them.

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expansion valve	an expandable chamber, associated with a fluid channel, which chamber (a) is filled with a gas or a liquid with a boiling point within about 10°C of the intended operating temperature of the liquid distribution system and (b) has an associated heater element for heating the expandable chamber to boil the liquid or expand the gas to cause sufficient expansion of the expandable chamber to fill a cross-section of the fluid channel.
• hole diameter	because techniques for fabricating small holes often create holes that are wider at one end than the other (for instance, about 50 microns wider), the hole diameter values recited to herein refer to the narrowest diameter.
• horizontal, vertical, EW, NS	indications of the orientation of a part of the distribution system refer to the orientation when the device is in use. The notations "EW axis" and "NS axis" are in reference to Figures 1, 2, 3 and 7, where an EW axis goes from right to left and is perpendicular to the long axis of the page and a NS axis is from top to bottom parallel to the long axis of the page.
• independent	channels, reservoirs or reaction cells that are not connected.
• offset	two sets of channels are "offset" when none of the channels in the first such set is adjacent to any of the channels in the second set.

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• open zone

a region of a channel that widens to decrease the flow-induced pressure of a fluid flowing through the channel.

• perpendicular

channels in the distribution plate are perpendicular even if primarily located on separate horizontal planes if their vertical projections onto the same horizontal plane are perpendicular.

• reservoir

unless a different meaning is apparent from the context, the terms "reservoir" and "fluid reservoir" include the horizontal extension channels (sometimes simply termed "extensions") directly connected to the reservoir or fluid reservoir.

• second reservoir
  extension channels

these extension channels include the distribution channels that may branch off of these extension channels.

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• substantially the
  length of one of the
  horizontal dimensions

at least about 70% of on of the major horizontal dimensions (e.g. the EW or NS dimensions illustrated in the Figures) of the distribution plate.

• U-plumbing channel   a channel designed to connect at least two
                            channels or reservoirs such that the liquid level
                            in one of the connected channels or reservoirs
                            will equalize with the liquid level in the other
                            connected channel or reservoirs due to
                            hydrological forces. U-plumbing channels
                            typically have vertical channels that connect
                            channels or reservoirs located in a higher vertical
                            plane with a substantially horizontal channel
                            segment of the U-plumbing channel located in a
                            lower plane -- these vertical and horizontal
                            segments together comprise the U-plumbing
                            channel. The feeder channels of the invention
                            are typically U-plumbing channels.

DETAILED DESCRIPTION

One version of the invention is a hydrologic liquid distribution
system comprising: a distribution plate comprising: a least two first

5   reservoirs having a common first fill level; at least one feeder channel
connected to and extending from each of the first reservoirs, each feeder
channel having a horizontal feeder channel segment, wherein the top of the
horizontal feeder channel segment is below the first fill level; at least one
distribution channel connected to each feeder channel having a second fill

10   level at substantially the same elevation as the first fill level; and a first liquid
level maintaining means for maintaining the liquid level in each first reservoir
at the first fill level, wherein filling each first reservoir to the first fill level
results in the filling of the connected feeder channels and filling the connected
distribution channels to the second fill level. The distribution channels

15   preferably have at least one capillary barrier.

The distribution plate is preferably coupled with a
feedthrough plate that fits above the distribution plate and provides channels
or conduits to allow for the passage of liquids or electrical leads through to
the distribution plate. A detachable reaction cell plate is preferably coupled

underneath the distribution plate and contains one or more, more preferably
two or more, reaction cells into which liquids from at least two reservoirs may
be distributed via the distribution plate. The reaction cell plate preferably
contains about 100 or more reaction cells, each addressable by at least two

5   reservoirs. More preferably the reaction cell plate contains about 1,000 or
more reaction cells, each addressable by at least two reservoirs. Yet more
preferably the reaction cell plate contains about 10,000 or more reaction
cells, each addressable by at least two reservoirs.

The distribution plate preferably has multiple first reservoirs.

10   The first reservoirs can have a primary reservoir portion and one or more first
reservoir extension channels, which preferably together extend along
substantially the length of one of the horizontal dimensions of the distribution
plate. Two or more such extended first reservoirs are preferably adjacent and
parallelly arrayed along one side of the distribution plate. In some

15   embodiments, it is desirable to relay liquid from two or more of such parallel,
extended first reservoirs to each of two or more reaction cells arrayed along a
substantially straight line (the "linear" reaction cells). To accomplish this
liquid relay, preferably a first feeder channel extends from the parallel first
reservoir nearest the edge of the distribution plate and underneath the

20   interiorly located parallel first reservoirs, while separate, feeder channels,
located adjacent to and parallel to the first feeder channel, extend from the
interiorly located parallel first reservoirs passing beneath other first reservoirs
as needed to maintain the segregation of the liquids in the reservoirs. In this
preferred embodiment, arrayed adjacent to the location of each of the linear

25   reaction cells are one distribution channel for each such first reservoir, each
such distribution channel connected to a separate one of the parallel feeder
channels. Thus, the distribution channels located adjacent to a reaction cell
form a "bundle" of distribution channels. Using pumps located in each of the
bundled distribution channels and conduits from each bundled distribution

30   channel to the adjacent reaction cell, liquid from each of the separate first
reservoirs can be distributed to any of the linear reaction cells.

Each distribution channel preferably includes at least one

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pump, which preferably comprises two or three electrodes. To operate the

pumps in an appropriate sequence, the distribution system of the invention is

preferably connected to a controller that controls each of the pumps. The

preferred two electrode pumps preferably are operated by applying a pulsed

5   voltage program appropriate for moving the liquid sought to be pumped.

Preferably, the controller includes a data storage device comprising a

database of pumping programs designating parameters, such as the pulse

length, voltage amplitude, pulse shape and voltage polarity, suitable for

moving each of a plurality of liquids.

10   The invention further relates to a preferential flow liquid

distribution system, which is suitable, for example, for conducting reactions

for chemical syntheses or chemical reactions involved in an assay, the

distribution system comprising: two or more continuous flow channels, each

having a upstream end and a downstream end, wherein the continuous flow

15   channels have an open zone at the downstream end;   for each continuous

flow channel, a branch channel extending off of that continuous flow channel

in the open zone; and, for each continuous flow channel, an alpha constrictor

for constricting flow in the open zone, the alpha constrictor located

downstream of the branch channel inlet, wherein when a liquid flows through

20   one of the continuous flow channels from the first to the second end a first

ratio amount of fluid is diverted into the branch channel when the alpha

constrictor is not operating and a second ratio amount, which is greater than

the first ratio amount, flows into the branch channel when the alpha

constrictor is in operation. Preferably, the branch channel inlets are within

25   about 300 microns of the upstream point at which the open zone begins.

Preferably, the distribution system has, for at least one branch channel, a beta

constrictor for constricting flow through the branch channel. In another

preferred embodiment, the distribution system has a buffer channel

addressable by at least two continuous flow channels via their associated

30   branch channels.

The invention additionally relates to a expansion valve liquid

distribution system made up of a reaction cell, two or more feeder channels, a

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separate conduit for each feeder channel connecting that feeder channel to
the reaction cell, and a expansion valve for each conduit, wherein the
expansion valve has an expanded state that fills a cross-section of the conduit
and prevents fluid flow through the conduit and an contracted state that

5   allows fluid flow through the conduit. In a preferred embodiment, conduits
have two or more, preferably three or more, expansion valves which can be
operated in concert to pump liquid from the connected feeder channel into the
reaction cell.

        The invention further relates to a electrode-based liquid

10   distribution system made up of (a) one or more, preferably two or more,
feeder channels each made up of a feeder channel inlet and a feeder channel
outlet and, connected to each such feeder channel, a distribution channel,
each feeder channel having a three-way junction connecting a feeder channel
inlet, a feeder channel outlet and the connected distribution channel, the

15   distribution channel connecting with a reaction cell, (b) for each such
three-way junction, a first electrode-based pump in the feeder channel inlet or
in the feeder channel outlet and (c) for each such three-way junction, a
second electrode-based pump in the distribution channel, wherein the
voltages applied to the first electrode-based pump and the second

20   electrode-based pump can be chosen (i) so that fluid in one of the feeder
channels can be moved from the feeder channel inlet to the feeder channel
outlet of the feeder channel without substantial flow into the connected
distribution channel or (ii) so that a substantial amount of flow proceeds via
the connected distribution channel.

25   A. A Basic Liquid Distribution System

        The invention relates to methods of addressing a large
number of reaction cells 350 with a plurality of fluid reservoirs 200 (see
Figures 1 and 2). In figure 1, reservoirs 200A - 200D are connected to
reservoir extension channels 212A - 212D via first connector channels 211A -

30   211D, respectively. The ceilings of first connector channels 211A - 211D are
located in a lower horizontal plane than the floors of extension channels 212A
- 212D, thereby assuring, for instance, that fluid from reservoir 200B does

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not leak into the extension channel 212A connected to reservoir 200A. Each first connector channel 211A - 211D connects with its respective reservoir extension 212A -212D via vertical channels (not illustrated). Connected to extension channels 212A - 212D are first, second, third, fourth and fifth sets

5   213A - 213E of first, second, third and fourth feeder channels 216A - 216D. The ceilings of these feeder channels are located in a horizontal plane beneath the floors of the extension channels 212A - 212D. Via these extension channels, fluid from each of the four first fluid reservoirs 200A - 200D can be brought to a location adjacent to any of the one hundred reaction cells 350

10   into which the fluid can be moved under the control of pumps or valves as described hereinbelow. Note that reaction cells 350 are located in a lower horizontal plane than first, second, third and fourth feeder channels 216A - 216D. Other geometries by which a large number of reaction cells can be addressed by separated fluid reservoirs are described below.

15   Features of other distribution systems described in this application can be applied to this embodiment, irrespective of under which subheading they are described. It will be understood by those of ordinary skill that while the embodiments of the invention are described with reference to channels that join at orthogonal angles, other angles are possible. In

20   preferred embodiments of the invention, the operational flow rate (i.e., the flow rate when the appropriate flow-inducing mechanisms are activated) from a given reservoir (e.g. first fluid reservoir 200) to a given reaction cell 350 is from about $0.01\mu l/$ min to about $10\mu l/$ min, more preferably from about $0.1\mu l/$ min to about $0.3\mu l/$ min.

25   **B. Hydrologic Liquid Distribution System**
     i. the distribution, feedthrough and reaction cell plates

                     Typically, the liquid distribution system of the invention will be formed of at least three plates, a feedthrough plate 300, a distribution plate 310 and a reaction cell plate 320. The feedthrough plate 300 is

30   typically bonded to the distribution plate 310 using one of the methods described below. The reaction cell plate 320 is typically removably fitted to the underside of the distribution plate 310, or the underside of intermediate

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plate 330 (not illustrated) interposed between the distribution plate 310 and the reaction cell plate 320.

Figure 2 shows the layout of a distribution plate 310 according to the invention. Figure 3 shows an expanded view of a portion of
5   a distribution plate 310 that better illustrates some of the features obscured by the scale of Figure 2. Typically, the structures indicated in solid lines will be formed in the top layer of the distribution plate 310, while the structures indicated with dotted lines will be formed in the bottom layer of the distribution plate 310, except that in Figure 2 the reaction cells 350 are
10 indicated by boxes in solid lines even thought these structures are located in a lower plane. Where appropriate, vertical channels connect the structures in the top of the distribution plate 310 with those in the bottom. For convenience, the axis from the top of the illustration to the bottom is designated the NS axis, while the axis from right to left is the EW axis.
15   At the top of Figure 2 are four first fluid reservoirs 200A, 200B, 200C and 200D, each having a defined fill level. Each of these first fluid reservoirs 200A, 200B, 200C and 200D has two first reservoir extensions 212 extending along substantially all of an EW axis of the distribution plate 310. The ceilings of the first reservoir extensions 212
20 preferably are at substantially the same elevation as the first fill level. At five staggered locations, A1, B1, C1, D1 and E1, along the EW axis of the first reservoir extensions 212 there are four first vertical channels 214 (see Figures 5 and 6B) that connect the first reservoir extensions 212 with four first horizontal feeder channel segments 216 that are formed in the bottom layer
25 of the distribution plate 310. At each staggered location A1, B1, C1, D1 or E1, four adjacent first horizontal feeder channel segments 216, which are connected to separate first reservoir extensions 212, extend along an NS axis to ten positions, A2, B2, C2, D2, E2, F2, G2, H2, I2 and J2. Each position A2, B2, C2, D2, E2, F2, G2, I2 or J2 along the course of each such set of
30 four adjacent horizontal feeder channel segments 216 is adjacent to a pair of reaction cells 350 (see Figures 5 and 6B). At these positions A2, B2, C2, D2, E2, F2, G2, H2, I2, or J2, the four adjacent first horizontal feeder channel

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segments 216 are separately connected, via separate second vertical
channels 225 (see Figures 4A, 4B, 5 and 6A), to each of four perpendicular
first distribution channels 222 formed in the top layer of the distribution plate
310. The ceilings of the first distribution channels 222 define a second fill

5   level that is typically substantially the elevation of the first fill level. The fill
level of a distribution channel (e.g., the second fill level) is "substantially" the
fill level of the connected reservoir (e.g., the first fill level) if they are offset
vertically by no more than about 10% of the depth of the channel; even if the
fill levels are further offset vertically they are still substantially the same if

10   filling the reservoir to its fill level results in filling the connected distribution
channel and the retention of fluid in the connected distribution channel (for
instance, retention due to the capillary barriers described further below with
reference to Figure 4A). The combination of a first vertical channel 214,
connected to a horizontal feeder channel segment 216, in turn connected to a

15   second vertical channel 225 makes up a first feeder channel 217 (not
identified in the Figures).

If liquids are maintained at a defined first level in a first fluid
reservoir 200, then substantially the same level will be maintained in the first
distribution channels 222 connected to that first fluid reservoir 200 via first

20   feeder channels 217. This equalization occurs due to the principle that two
connected bodies of liquid will tend to seek the same level and, where the
size of the channels allows, due to capillary flow. Liquids are maintained at a
defined level in the first fluid reservoirs. In the illustrated embodiment, liquid
is fed into the fluid reservoir 200 through channels in the feedthrough plate

25   300 and such liquid that is not needed to fill the fluid reservoirs to the defined
level is drained through drains 380. First openings 381 (not shown) are
formed in the bottom layer of the feedthrough plate 300 to create a liquid
connection or sluice between the first fluid reservoirs 200 and the drains 380.
Liquids are constantly feed into the first fluid reservoirs 200 (as well as the

30   second fluid reservoirs 210 and third fluid reservoirs 220) typically by the use
of an external pump 15 (not shown), such as the model number 205U
multichannel cassette pump available from Watson-Marlow, Inc.

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Alternatively, a defined level can be maintained by monitoring the level of liquid in the first fluid reservoirs 200 (or second fluid reservoirs 210 or third fluid reservoirs 220) and only activating the pumps feeding liquid to a given fluid reservoir when needed to maintain the defined level.

5   Each set of four adjacent first distribution channels 222 are adjacent to two buffer channels 218, located to each side of the first distribution channels 222 along the EW axis. Liquid can be pumped from any first distribution channel 222 into the adjacent buffer channel 218 by activating the first pump 360 (indicated in Figure 3 by two filled dots

10 representing the electrodes of one type of pump) of the first distribution channel 222. This pumping creates additional pressure that moves the liquid over capillary barrier 370 (see Figures 4A, 4B and 5) separating the first distribution channel 222 and the buffer channel 218. Between each first distribution channel 222, second distribution channel 224 or third distribution

15 channel 226 and the adjacent buffer channel 218 and between each buffer channel 218 and its adjacent third vertical channel 390 (described below) there is such a capillary barrier 370 that inhibits liquid flow when the pumps are not activated. Second openings 362 (see Figures 4A and 4B) are formed in the bottom layer of the feedthrough plate 300 to create a liquid connection

20 or sluice between the first distribution channels 222 and the buffer channels 218. From a buffer channel 218, liquid can be pumped using a second pump 361 (indicated in Figure 3 by two filled dots representing the electrodes of one type of pump) to a third vertical channel 390 that connects with a reaction cell in the reaction cell plate 320. Third openings 363 (see Figures

25 4A and 4B) in the bottom layer of the feedthrough plate 300 or the distribution plate 310 serve to create a liquid connection or sluice between the buffer channels 218 and third vertical channels 390.

Figure 4A illustrates a capillary barrier 370, at which a meniscus 371 forms, at the junction between a first distribution channel 222

30 containing liquid 11 and either a buffer channel 218 or a third vertical channel 390. The meniscus 371 formed at the outlet of first distribution channel 222 into buffer channel 218 will tend to inhibit seepage from the first distribution

channel 222, such as the seepage that can result from capillary forces.
Figure 4B shows, in a variation of the distribution system illustrated in Figures
2 and 3, capillary barriers 370 at the junction between first distribution
channel 222 and buffer channel 218 and at the junction between buffer

5   channel 218 and third vertical channel 390. Also shown are first electrode
360A and second electrode 360B making up first pump 360 and third
electrode 361A and fourth electrode 361B making up pump 361. In some
embodiments there are vents (not illustrated) that extend through the
feedthrough plate 300 at the tops of buffer channel 218 or third vertical

10   channel 390.

Note that only a small cut-away of NS oriented horizontal
feeder channel segments 216 are shown in Figures 4A and 4B. Typically,
these channels extend inwardly and outwardly from the illustrated cut-away
and connect with additional first distribution channels 222 situated to

15   distribute liquid to other reaction cells 350.

Along the right side of the distribution plate 310 are ten
second fluid reservoirs 210, each having a second reservoir extension 240
extending along an EW axis. Second distribution channels 224 form
"L"-extensions off of second reservoir extensions 240 and are each

20   positioned adjacent to a separate buffer channel 218, such that there are ten
second distribution channels 224 extending off of each second reservoir
extension 240. Each second distribution channel 224 has a pump 360 that
can move liquid from a second distribution channel 224 to the adjacent buffer
channel 218. Second openings 362 (not shown) in the bottom of

25   feedthrough plate 300 serve to provide a sluice or route of liquid connection
between the second distribution channels 224 and the buffer channels 218.
Liquid moves from the buffer channels 218 to the reaction cells as described
above. Located adjacent to each second reservoir 210 is a drain 380 (not
shown) that operates to maintain a defined third fill level as described above.

30   As will be described further below in Section H in reference
to Figures 16A - 16D, the capillary barriers 370 and sluices created by the
second openings 362 or third openings 363 act as a combined valve and

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Third vertical channel 390 connects buffer channel 218 to reaction cell 350,
which has a drain 355.

Figure 6B shows a vertical cross-section along an EW axis
that slices through a first fluid reservoir 200. From first fluid reservoirs
5 extensions 212 can be seen two first vertical channels 214 that each connect
to a separate horizontal feeder channel segment 216. Drain 380 for first fluid
reservoir 200 is indicated by dotted lines. In the illustrated distribution
system, first fluid reservoir 200 opens to the top of feedthrough plate 300 via
fourth openings 302.

10   The operation of the liquid distribution system can be
described with reference to Figure 7. Liquid can be distributed to first
reaction cell 350AA (not shown) connected to third vertical channel 390A
from any of first, second or third fluid reservoirs 200A, 200B, 200C, 200D,
210A or 220A. If "liquid A" is maintained at first fluid level in fluid reservoir
15 200A, liquid A will flow through the connected vertical channels 214 into the
connected horizontal feeder channel segments 216A, 216A2, 216A3, 216A4
and 216A5 and into the connected distribution channels 222A1, 222A2, and
so on. From first distribution channel 222A1, liquid A is pumped into first
buffer channel 218AA using pump 360A under the control of controller 10
20 (not shown). From first buffer channel 218AA, liquid A is pumped into first
reaction cell 350AA via third vertical channel 390A using pump 361A under
the control of controller 10.

When a distribution plate 310 according to the invention
makes use of a buffer channel 218 and the buffer channel 218 lacks a vent to
25 the outside atmosphere, the relationship between the pumping rate of a first
pump 360 that pumps liquid into the buffer channel 218 and the pumping
rate of a second pump 361 that pumps liquid from the buffer channel 218 to
a reaction cell 350 can be manipulated to advantage. When a first pump 360
moves liquid from, for instance, distribution channel 222A1 (Figure 7), a
30 pressure increase is created in buffer channel 218 which inhibits flow past the
capillary barriers for distribution channels 222B1, 222C1, 222D1, 224AA and
226AA. A delay in the corresponding activation of second pump 361 will

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pump. The capillary barriers 370 prevent flow to the reaction cell, which flow would be favored by capillary forces, until the first pumps 360 or second pumps 361 provide the extra pressure needed to overcome the capillary barriers 370. Narrowing the sluices can increase the capillary forces favoring

5   flow, thereby reducing the amount of added pressure needed to overcome the capillary barriers 370. The use of the capillary barriers 370 allows flow control to be governed by the first pumps 360 or second pumps 361, which are typically controlled by controller 10.

Located along the bottom edge of the distribution plate

10 illustrated in Figure 1 are ten third liquid fluid reservoirs 220. Horizontal feeder channel segments 230 are connected to the third fluid reservoirs 220 and to third distribution channels 226 via fourth vertical channels 227. The third distribution channels 226 have first pumps 360 which can move liquid into adjacent buffer channels 218 via openings 362 (not shown) in the

15 feedthrough plate 300. Located adjacent to each third fluid reservoir 220 is a drain 380 (not shown) that operates to maintain a defined fourth fill level as described above. Third fluid reservoirs 220 and connected third distribution channels 226 operate in just the same way as first fluid reservoirs 200 and first distribution channels 222. Those of ordinary skill in the art will readily

20 envision alternative geometries wherein a number of separate third fluid reservoirs 220 can interact with a given buffer channel 218 via a number of third distribution channels 226 positioned adjacent to the buffer channel 218. Located adjacent to each third reservoir 220 is a drain 380 (not shown) that operates to maintain a defined third fill level as described above.

25   Figure 5 shows perspective views of portions of feedthrough plate 300, distribution plate 310 and reaction cell plate 320.

        Figure 6A shows a vertical cross-section along an EW axis that slices through a first distribution channel 222. Underneath the first distribution channel 222 can be seen four horizontal feeder channel segments

30 216, only one of which is connected to the first distribution channel via visible second vertical channel 225. Leads 303 and 304 which connect the electrodes of the first pumps 360 and second pumps 361 are illustrated.

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result in this cross-contamination inhibiting pressure being maintained. The buffer channel 218 further serves to dilute any accidental overflows from distribution channels containing reagents not intended for the adjacent reaction cell 350. This dilution will typically reduce the concentration of

5 reactive reagents beneath the concentration effective in the reaction process that is intended for the adjacent reaction cell 350.

Note that in the illustrated embodiment, each of six solutions or solvents can be distributed to each of one hundred reaction cells 350 from first, second and third fluid reservoirs 200A, 200B, 200C, 200D, 210 and

10 220. Each of the ten second fluid reservoirs 210, or each of the ten third fluid reservoirs 220, can contain a separate solution or solvent intended for use in the ten associated reaction cells that can be addressed by the fluid reservoir. The four first fluid reservoirs 200A, 200B, 200C and 200D each can address any of the one hundred reaction cells 350. Further flexibility is

15 available by flushing a given fluid reservoir with a new solution or solvent when appropriate during a protocol.

Note that the buffer channels 218 are optional. Buffer channels 218 can be avoided by providing for direct conduits from the outlets of distribution channels (which outlets are preferably capillary barriers 370) to

20 the appropriate reaction cell 350.

The fluid reservoirs (e.g. first, second and third fluid reservoirs 200, 210 and 220) are typically simply expanded (i.e. wider) portions of the attached extension channels. Preferably, the liquid in the fluid reservoirs is maintained within ± 10 % of the difference between the floor of

25 the fluid reservoir and its fill level. Replenishment of the liquid in the reservoirs can be continuous, step-wise on a defined schedule, or based on sensor data feed to controller 10. The drains 380 are designed to remove any excess fluid added to the reservoir by use of external pump 15. The fluid reservoirs, filled to the respective fill level, preferably have a volume from

30 about 1 μl to about 5 μl, more preferably about 02.5 μl. Thus, in the more preferred embodiment, the volume in the fluid reservoirs will preferably be 2.5 μl ± 0.25 μl. Appropriate peristaltic pumps, such as the model number 205U

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multichannel cassette pump available from Watson-Marlow, Inc., can deliver
liquid at rates as low as 1 μl per second. Such pumps need only be activated
for a fraction of a second to adequately recharge a fluid reservoir.

The above discussion describes the distribution system as
5   being formed with a feedthrough plate 300, distribution plate 310 and
reaction cell plate 320. However, it will be clear that additional plates can
conveniently be incorporated into the distribution system. For instance, a
intermediate plate 330 is, in a preferred embodiment, permanently bonded
underneath the distribution plate 310 and interposed between the distribution
10   plate 310 and the reaction cell plate 320. The use of the intermediate plate
330 allows for much greater flexibility in the design of the channels the form
the distribution system.

ii. the pumps

15   Any pumping device of suitable dimensions can be used as
the internal first pumps 360 or second pumps 361 in the liquid distribution
system of the invention. Such pumps can include microelectromechanical
systems (MEMS) such as reported by Shoji et al., "Fabrication of a Pump for
Integrated Chemical Analyzing Systems," *Electronics and Communications in*
20   *Japan*, Part 2, 70: 52-59, 1989 or Esashi et al., "Normally closed microvalve
and pump fabricated on a Silicon Wafer," *Sensors and Actuators*, 20:
163-169, 1989 or piezo-electric pumps such as described in Moroney et al.,
"Ultrasonically Induced Microtransport," *Proc. MEMS*, 91: 277-282, 1991.
Preferably, however, the first pumps 360 and second pumps 361 have no
25   moving parts. Such first pumps 360 or second pumps 361 can comprise
electrode-based pumps. At least two types of such electrode-based pumping
has been described, typically under the names "electrohydrodynamic
pumping" (EHD) and "electroosmosis" (EO). EHD pumping has been
described by Bart et al., "Microfabricated Electrohydrodynamic Pumps,"
30   *Sensors and Actuators*, A21-A23: 193-197, 1990 and Richter et al., "A
Micromachined Electrohydrodynamic Pump," *Sensors and Actuators*,
A29:159-168, 1991. EO pumps have been described by Dasgupta et al.,

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"Electroosmosis: A Reliable Fluid Propulsion System for Flow Injection

Analysis," *Anal. Chem.*, 66: 1792-1798, 1994.

EO pumping is believed to take advantage of the principle

that the surfaces of many solids, including quartz, glass and the like, become

5 charged, negatively or positively, in the presence of ionic materials, such as

salts, acids or bases. The charged surfaces will attract oppositely charged

counter ions in solutions of suitable conductivity. The application of a voltage

to such a solution results in a migration of the counter ions to the oppositely

charged electrode, and moves the bulk of the fluid as well. The volume flow

10 rate is proportional to the current, and the volume flow generated in the fluid

is also proportional to the applied voltage. Typically, in channels of capillary

dimensions, the electrodes effecting flow can be spaced further apart than in

EHD pumping, since the electrodes are only involved in applying force, and

not, as in EHD, in creating charges on which the force will act. EO pumping

15 is generally perceived as a method appropriate for pumping conductive

solutions.

EHD pumps have typically been viewed as suitable for

moving fluids of extremely low conductivity, e.g., 10^{-14} to 10^{-9} S/cm. It has

now been demonstrated herein that a broad range of solvents and solutions

20 can be pumped using appropriate solutes than facilitate pumping, using

appropriate electrode spacings and geometries, or using appropriate pulsed or

d.c. voltages to power the electrodes, as described further below.

The electrodes of first pumps 360 and second pumps 361

used in the liquid distribution system preferably have a diameter from about

25 25 microns to about 100 microns, more preferably from about 50 microns to

about 75 microns. Preferably, the electrodes protrude from the top of a

channel to a depth of from about 5 % to about 95 % of the depth of the

channel, more preferably from about 25 % to about 50 % of the depth of the

channel. Usually, as a result the electrodes, defined as the elements that

30 interact with fluid, are from about 5 microns to about 95 microns in length,

preferably from about 25 microns about to 50 microns. Preferably, a pump

includes an alpha electrode 364 (such as first electrode 360A or third

24 .

electrode 361A) and a beta electrode 365 (such as third electrode 360B and

fourth electrode 361B) that are preferably spaced from about 100 microns to

about 2,500 microns apart, more preferably, from about 250 microns to

about 1000 microns apart, yet more preferably, from about 150 microns to

5   about 250 microns apart. The separation of electrodes is measured from the

center points of the electrodes as they first protrude into their associated fluid

channel. In a particularly preferred embodiment, a gamma electrode 366 (not

shown) is spaced from about 200 microns to about 5,000 microns, more

preferably from about 500 microns to about 1,500 microns, yet more

10 preferably about 1,000 microns from the farther of the alpha electrode 364

and the beta electrode 365. In an alternative preferred embodiment, the

pump has two additional electrodes comprising a gamma electrode 366 (not

shown) and a delta electrode 367 that are spaced from about 200 microns to

about 5,000 microns, more preferably from about 500 microns to about

15 1,500 microns, yet more preferably about 1,000 microns apart. Where the

electrodes are located in fluid channels that have bends, the distances are

measured along a line that defines the center line of the fluid channel. In

contexts where relatively low conductivity fluids are pumped, voltages are

applied across the alpha electrode 364 and the beta electrode 365, while in

20 contexts where relatively more highly conductive fluids are pumped the

voltage is induced between gamma electrode 366 and one of alpha electrode

364, beta electrode 365 or delta electrode 367. The latter circumstance

typically applies for solvents traditionally pumped with EO pumping, although

this invention is not limited to any theory that has developed around the

25 concepts of EHD or EO pumping. No firm rules dictate which electrode

combination is appropriate for a given solvent or solution; instead an

appropriate combination can be determined empirically in light of the

disclosures herein.

The voltages used across alpha and beta electrodes 364 and

30 365 when the pump is operated in d.c. mode are typically from about 50 V to

about 2,000 V, preferably from about 100 V to about 750 V, more preferably

from about 200 V to about 300 V. The voltages used across gamma

electrode 366 and alpha, beta or delta electrodes 364, 365 or 367 when the
pump is operated in d.c. mode are typically from about 50 V to about 2,000
V, preferably from about 100 V to about 750 V, more preferably from about
200 V to about 300 V. The voltages used across alpha and beta electrodes

5   364 and 365 when the pump is operated in pulsed mode are typically from
about 50 V to about 1,000 V, preferably from about 100 V and about 400 V,
more preferably from about 200 V to about 300 V. The voltages used across
gamma electrode 366 and the alpha, beta or gamma electrode 364, 365 or
367 when the pump is operated in pulsed mode are typically from about 50 V

10   to about 1,000 V, preferably from about 100 V and about 400 V, more
preferably from about 200 V to about 300 V. Preferably, the ratio of
pumping to current will be such that no more than about one electron flows
into the solution adjacent to a first pump 360 or second pump 361 for every
1,000 molecules that move past the pump 360 or 361, more preferably for

15   every 10,000 molecules that move past the pump 360 or 361, yet more
preferably for every 100,000 molecules that move past the pump 360 or
361.

It is believed that an electrode-based internal pumping
system can best be integrated into the liquid distribution system of the

20   invention with flow-rate control at multiple pump sites and with relatively less
complex electronics if the pumps are operated by applying pulsed voltages
across the electrodes. Figure 8 shows an example of a pulse protocol where
the pulse-width of the voltage is r_1 and the pulse interval is r_2. Typically, r_1
is between about 1 μs and about 1 ms, preferably between about 0.1 ms and

25   about 1 ms. Typically, r_2 is between about 0.1 μs and about 10 ms,
preferably between about 1 ms and about 10 ms. A pulsed voltage protocol
is believed to confer other advantages including ease of integration into high
density electronics (allowing for hundreds of thousands of pumps to be
embedded on a wafer-sized device), reductions in the amount of electrolysis

30   that occurs at the electrodes, reductions in thermal convection near the
electrodes, and the ability to use simpler drivers. The pulse protocol can also
use pulse wave geometries that are more complex than the block pattern

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illustrated in Figure 8.

Another, procedure that can be applied is to use a number of
electrodes, typically evenly spaced, and to use a travelling wave protocol that
induces a voltage at each pair of adjacent electrodes in a timed manner that
5   first begins to apply voltage to the first and second electrodes, then to the
second and third electrodes, and so on. Such methods are described in Fuhr
et al., *J. Microelectrical Systems*, 1: 141-145, 1992. It is believed that
travelling wave protocols can induce temperature gradients and corresponding
conductivity gradients that facilitate electric field-induced fluid flow. Such
10   temperature gradients can also be induced by positioning electrical heaters in
association with the electrode-based first pumps 360 and second pumps 361.

While not wishing to be restricted to theory, several
theoretical concepts are believed to play a role in the mechanics of EHD
pumping. The forces acting on a dielectric fluid are believed to be described
15   by:

$$\vec{F} = q\vec{E} + \vec{P} \cdot \nabla \vec{E} - \tfrac{1}{2}E^1\nabla\varepsilon + \nabla\left[\tfrac{1}{2}\rho\frac{\partial\varepsilon}{\partial\rho}E^2\right]$$

where F is the force density, q is the charge density, E is the applied field, P
20   is the polarization vector, ε is the permittivity and ρ is the mass density. Of
the terms in the equation, the first and third are believed to be the most
significant in the context of EHD pumping of fluids. The first term (qE) relates
to the Coulomb interaction with a space-charge region. The third term
($\tfrac{1}{2}E^2\nabla\varepsilon$) relates to the dielectric force which is proportional to the gradient in
25   permittivity.

In low fields, i.e., the Ohmic region where current is linearly
proportional to voltage, the primary source of charges that will be acted upon
by the electric field are believed to be primarily due to ions from additives,
ions from impurities and ions formed by autodissociation of molecules in the
30   fluid. In intermediate fields, i.e. from beyond the Ohmic region to about 2
V/μm, the charges are believed to be primarily formed by dissociation and

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electrolytic processes in the fluid. In higher fields, the charges are believed to be determined by injection processes at the electrodes, which electrodes inject homocharges.

For the purposes of this application, positive (+) flow shall
5   be flow in the direction of the negative electrode, and negative (-) flow shall be flow in the direction of the positive electrode.

In a preferred embodiment of the invention, the controller 10 has a device for storing data and stores the values of voltage and polarity suitable for pumping a number of solvents.

10   Experimental results indicate that the properties of fluid flow (like direction of flow) correlate well with the solvent's ability to stabilize and solvate the charged species injected or induced from the electrodes. The direction of flow is believed to be determined by the preference of the solvent to solvate either positive charges or negative ions. This solvation preference
15   is believed to imply a greater shell of solvent molecules that will be dragged in an electric field, creating fluid movement, when a field is applied to the electrodes of a first pump 360 or a second pump 361. For example, a preferred solvation of positive charges correlates with a preference for fluid flow from the anode to the cathode (i.e., the positive direction). The degree
20   of such a solvation preference for a solvent is believed to depend on the ability of the molecules within the solvent to accept or donate hydrogen bonds. In one aspect of the invention, for liquids whose pumping behavior has not yet been characterized, the controller will store initial pumping parameters estimated using on the Linear Solvation Energy relationships
25   established by R.W. Taft and co-workers. See, Kamlet et al., *J. Org. Chem.*, 48: 2877-2887, 1983 and Kamlet et al., *Prog. Phys. Org. Chem.*, 13: 485, 1981. These workers have categorized solvents in terms of the following parameters: π, the ability of the solvent to stabilize a stabilize a charge or dipole by virtue of its dielectric properties; α, the hydrogen bond donating
30   ability of the solvent; and β, the hydrogen bond accepting ability of the solvent. These parameters are more fully defined in the above-cited Kamlet et al. publications.

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Using a one mm capillary of circular cross-section, a pair of
50 micron rod-shaped, platinum electrodes perpendicularly inserted to a depth
of 500 microns into the capillary with a 500 micron separation powered by a
400 V field, the direction of flow was determined for several solvents. The
direction of flow and the α, β, π, ϵ and dipole moment values are as follows:

Solvent	direction	α	β	π	ϵ	dipole moment
ethanol	-	0.83	0.77	.54	24.55	1.69
tetrahydro-furan	+	0	0.55	.58	7.58	1.75
chloroform	-	0.44	0	.58	4.806	1.01
acetone	+	0.08	0.48	.71	20.7	2.69
methanol	-	0.93	0.62	.6	32.7	2.87
2-propanol	+/-	0.76	0.95	.48	19.92	1.66
acetonitrile	+	0.19	0.31	.75	37.5	3.92
N-methyl-pyrrolidone	+	0	0.77	.92	32.0	4.09
diethyl ether	+	0	0.47	0.27	4.335	1.15
1,2 dichloro ethane	-	0	0	0.81	10.36	1.2
DMF	+	0	0.69	.88	36.71	3.86

It is believed that the α and β values reflect the ability of the
solvent under an electric field to solvate a negative or positive charged
species, with the magnitude of α - β correlating with (-) flow, and the
magnitude of β - α correlating with (+) flow. According to one aspect of the
invention, the preferred direction of flow of a liquid can be reversed from that
predicted as above if the fluid has a difference in α and β values that is small
but not zero and the electrode pair used creates an asymmetric field, such
that the acting force on either positive or negative charged species is
enhanced. One such electrode pair has an alpha electrode 364 with a sharp
point pointing in the direction of intended flow and a beta electrode 365 that

lines the walls of the channel in which it is located. Such an electrode-based pump, fabricated in a 1 mm capillary, has been shown to be effective to pump 2-propanol in the direction pointed to by the alpha electrode 364 either when the voltage applied to the electrodes implied a (-) direction of flow or,

5   with somewhat weaker flow, when the voltage applied to the electrodes implied a (+) direction of flow.

The pumping parameters of a liquid can be calibrated using a plug of the liquid disposed in a capillary that has an electrode-based pump and is angled uphill. If optical devices are associated with the capillary for

10   monitoring the position of the plug, the velocity of pumped flow uphill and the velocity of gravity driven downhill motion can be measured. With these velocities and the angle of the capillary, the pressure applied to the liquid can be calculated. (Fluid resistance, $R = (8 \bullet \mu \bullet l)/\pi r^4$, where μ defines viscosity and l = the length of the fluid plug; Pressure, $P = RA(v_{up} - v_{down})$, where A

15   = cross-sectional area). The efficiency of the pump can also be calculated ($\eta = (q \bullet p \bullet Q \bullet N_A)/m \bullet l$, where q = charge of e⁻, p = density of liquid, Q = flow rate = $v_{up} \bullet A$, m = mass of liquid, and l = current). The velocities can be measured with multiple single point observations of the location of either the front or rear interfaces of the plug using fixed LEDs and optical detectors or in

20   a continuous mode using a light and a silicon photodiode position sensor, such as a SL15 or SC10 position sensor available from UDT Sensors, Inc., Hawthorne, CA. With the latter method, the correlation between the signal produced at the difference amplifier connected to the position sensor must be calibrated prior to experimental use.

25   The pumping parameters for a number of solvents have been determined in the 1 mm capillary described above, as follows:

30

Solvent	Flow rate, Q $\mu l/sec$	Pressure, P N/m^2	electrical efficiency, η, molecules/e⁻
acetone	14.56	16.33	1.9×10^6
methanol	24.46	26.32	9.7×10^4
1-propanol	16.39	74.89	4.2×10^5
diethyl ether	18.44	20.45	5.8×10^8
1,2 dichloroethane	14.24	46.55	2.9×10^7

Another aspect of pumping is the observation that fluids that are resistant to pumping at a reasonable field strength can be made more susceptible to electrode-based pumping by adding a suitable flow-enhancing additive. Preferably, the flow-enhancing additive is miscible with the resistant fluid and can be pumped at high pressure, P, high flow rate, Q, and good electrical efficiency, η (i.e., molecules pumped per electron of current). Generally, the flow-enhancing additive comprises between about 0.05 % w/w and about 10 % w/w of the resistant fluid, preferably between about 0.1 % w/w and about 5 % w/w, more preferably between about 0.1 % w/w and about 1 % w/w. Carbon tetrachloride and cyclohexane do not pump using the electrode pump situated in a capillary described above at a voltage of 2,000 V. By adding 0.5 % w/w acetone or methanol as a flow-enhancing additive, both of these fluids can be pumped at a voltage of 1,000 V. In some cases, it is desirable to reverse the preferred flow direction of a liquid by mixing with it a flow-enhancing additive that strongly pumps in the desired direction. In all cases, additives are selected on the basis of their pumping characteristics and their compatibility with the chemistries or other processes sought to be achieved in the liquid distribution system.

The electrode-based pumps of the invention can be operated to as a valve to resist flow in a certain direction by operating the pumps to counter the unwanted flow. To power the electrode-based pumps, one or more digital drivers, consisting of, for example, a shift register, latch, gate and switching device, such as a DMOS transistor, permits simplified electronics so that fluid flow in each of the channels can be controlled

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independently. Preferably, each digital driver is connected to multiple switching devices that each can be used to control the pumping rate of a separate electrode-based pump.

Features of other distribution systems described in this
5   application can be applied to this embodiment, irrespective of under which subheading they are described.

C. Preferential Flow Liquid Distribution System

Another embodiment of the distribution system wherein a plurality of microscaled reaction cells can be separately addressed by a
10 number of reagent liquids uses a continuous flow system is illustrated schematically in Figure 9. Features of other distribution systems described in this application can be applied to this embodiment, irrespective of under which subheading they are described.

Figure 9 illustrates a first continuous flow channel 400A, a
·15 second continuous flow channel 400B, a third continuous flow channel 400C and a fourth continuous flow channel 400D, each continuous flow channel having an upstream end U and a downstream end D. Fluid can be pushed through first through fourth continuous flow channels 400A-400D with the assistance of one or more external pumps 171. Third pumps 460 (not
20 shown), which can be electrode-based pumps, can provide further pumping within the first through fourth continuous flow channels 400A-400D. Constricted zones 401A-401D at the upstream end of the first through fourth continuous flow channels 400A-400D have a first cross-sectional area. Open zones 402A-402D at the downstream end of the first through fourth
25 continuous flow channels 400A-400D have a second cross-sectional area that is greater than the first cross-sectional area. At the upstream end of the open zones 402A-402D, are first branch channel 410A, second branch channel 410B, third branch channel 410C and fourth branch channel 410D, respectively. Each of these branch channels 410A-410D preferably has a
30 smaller cross-sectional area than that of the open zones 402A-402D and each flows into a buffer channel 440. In the open zones 402A-402D, there are first alpha constrictor 420AA, second alpha constrictor 420AB, third alpha

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constrictor 420AC, and fourth alpha constrictor 420AD, respectively.

A constrictor, such as an alpha constrictor, can be a
micromachined valve or transducer that is thermally, electromagnetically or
pressure actuated, an electrode-based pump operating in flow-blocking mode,

5   or another constrictor recognized in the art. Constrictors operate to reduce
flow at a portion of a channel, or, preferably, to shut off flow.

The downstream outlets of the continuous flow channels
400A-400D connect with a common bypass 430 which connects to first
drain 431A and second drain 431B.

10   Branch channels 410A-410C have a first beta constrictor
420BA, second beta constrictor 420BB, third beta constrictor 420BC and
fourth beta constrictor 420BD, respectively. From buffer channel 440, there
extends a reaction cell conduit 442, a first buffer overflow 441A and a
second buffer overflow 441B. Reaction cell conduit 442 has a gamma

15   constrictor 420C. First and second buffer overflows 441A and 441B have
first delta constrictor 420DA and second delta constrictor 420DB,
respectively.

When a "liquid A" flows through first continuous flow
channel 400A and first alpha constrictor 420AA is not in operation, liquid A

20   will tend to flow to the common bypass 430 via the open zone portion of the
first continuous flow channel 400A having relatively large cross-sectional area
rather than flow through relatively narrow branch channel 410A. First beta
constrictor 420BA can be operated to restrict or block flow in branch channel
410A, further assuring greater flow through to the common bypass 430. To

25   direct liquid A to the reaction cell 350, first alpha constrictor 420AA is
operated, while first beta constrictor 420BA is not, resulting in greater flow
into first branch channel 410A. The entry of liquid A into buffer channel 440
can result in the application of upward pressure at second through fourth
branch channels 410B-410D, depending upon the rate of outflow through

30   reaction cell conduit 442 or first or second buffer overflows 441A or 441B.
This pressure can be used to inhibit cross contamination with fluids from
second, third or fourth continuous flow channels 400B-400D.

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All constrictors 420 preferably can operate to stop liquid flow in their respective channels, particularly first through fourth beta constrictors 420BA through 420BD and gamma constrictor 420C.

Figure 10A shows a cross-sectional view of continuous flow
5   channel 400A operated to direct flow into branch channel 410A, while Figure 10B illustrates the continuous flow channel 400A operated to direct flow into the common bypass 430. A reaction cell 350 and a reaction cell drain 355 are also illustrated.

Figure 11 shows how the first through fourth continuous
10   flow channels 400A-400D can be connected to a larger grid having first through fourth primary feed channels 470A-470D. First through fourth primary feed channels 470A-470D are located on a higher or lower horizontal plane than first through fourth continuous flow channels 400A-400D, allowing the two sets of channels to be interconnected. In one preferred
15   embodiment, the inlets of each of first through fourth continuous flow channels 400A-400D have third pumps 460 (not illustrated) that facilitate drawing fluid from the first through fourth primary feeder channels 470A-470D.

D. Expansion Valve Liquid Distribution System

20   . The expansion valve liquid distribution system has a reaction cell, two or more feeder channels, a separate conduit connecting each feeder channel to the reaction cell, and a expansion valve for each conduit, wherein the expansion valve has an expanded state that fills a cross-section of the conduit and prevents fluid flow through the conduit and an contracted state
25   that allows fluid flow through the conduit. This embodiment differs from other embodiments in that the distribution system is preferably constructed of plastic, rather than glass or a silicon-based material. Preferred plastics include polyethylene, polypropylene, liquid crystal engineering plastics, polyvinylidine fluoride and polytetrafluoroethylene. Plastics with low moisture vapor
30   transmission rates (e.g., polyethylene, polyvinylidine fluoride and polytetrafluoroethylene) are particularly preferred. Laminates such as a laminate of polyethylene and a polyester such as poly(ethyleneterephthalate)

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are also preferred for their vapor barrier properties. The channels or conduits

of this embodiment are preferably as described below in Section I, which

describes fabrication methods. However, this embodiment can more readily

be used with larger scale features, such as larger channels and reaction cells.

5   Features of other distribution systems described in this application can be

applied to this embodiment, irrespective of under which subheading they are

described.

Figure 12 shows a schematic having fifth through eighth

primary supply channels 580A through 580D, respectively. Fifth primary

10   supply channel 580A connects to first alpha feeder channel 570A1, second

alpha feeder channel 570A2, and so on. Sixth through eighth primary supply

channels, 580B through 580D, respectively, are also connected to feeder

channels. Focusing on second alpha feeder channel 570A2, second beta

feeder channel 570B2, second gamma channel 570C2 and second delta

15   feeder channel 570D2, these are each connected to a number of alpha   ·

distribution channels 500A, beta distribution channels 500B, gamma ·

distribution channels 500C and delta distribution channels 500D, respectively.

For instance, second alpha feeder channel 570A2 is connected to eleventh

alpha distribution channel 500A11, twelfth alpha distribution channel

20   500A12, and so on. Sets of four distribution channels 500, e.g. eleventh

alpha distribution channel 500A11, eleventh beta distribution channel

500B11, eleventh gamma distribution channel 500C11, and eleventh delta

distribution channel 500D11, are connected to a given reaction cell 350, e.g.,

reaction cell 350.

25   As illustrated below, each distribution channel 500 has an

expansion valve which can be activated to block flow from the feeder

channels 570 into the reaction cell 350 connected via the distribution channel

500. In one preferred embodiment, fluid in the primary supply channels 580

and feeder channels 570 is maintained a constant pressure using upstream

30   pumps and downstream pressure release valves.

Figure 13A shows a cross-section through eleventh alpha

distribution channel 500A11. Three of the plates that form the distribution

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system, first plate 591, second plate 592 and third plate 593, are illustrated.
Second alpha feeder channel 570A2, second beta feeder channel 570B2,
second gamma feeder channel 570A2 and second delta feeder channel 570
D2 can be formed in a molding process used to form first plate 591. Eleventh
5   alpha distribution channel 500A11 is primarily formed with parts of first plate
591 and second plate 592 and can be formed during the molding process
used to form these plates. The portion 501A11 of eleventh alpha distribution
channel 500A11 connecting to second alpha feeder channel 570A2 can be
formed using a drilling process, such as a laser drilling process. The portion
10 502A11 (see Fig. 13B) of eleventh alpha distribution channel 500A11 that
connects to reaction cell 350B1 is typically formed during the molding of
second plate 592. Expansion valve 580 includes a low modulus, elastomeric
film 581 such as a hydrocarbon elastomer, acrylonitrile-based elastomer or
polyurethane films, which films include natural latex films, ethylene-propylene
15 rubber and acrylonitrile-butadiene-styrene copolymer films. The elastomeric
film can, for example, be bonded to the substrate using an adhesive such as a
thermal setting acrylic, polyurea or polysulfide adhesive or it can be bonded
by thermal compression bonding or ultrasonic welding. Elastomeric film 581
covers a fluid chamber 582 that is filled with a gas, such as air or argon, or
20 with a low-boiling liquid, such as freon or another refrigerant. Situated
sufficiently near fluid chamber 582 is an heating element 583, which is
preferably controlled by controller 10. The heating element 583 functions to
heat the gas or liquid in fluid chamber 582 to cause the expansion of the
expansion valve 580. Reaction cell 350B1 has a drain 355B1.
25   Heating elements 583 can be any number of heating devices
known to the art including electrical resistance heaters and infrared light
sources, including infrared diode lasers, such as edge-emitting diode laser
arrays available from David Sarnoff Research Center, Princeton, NJ or the
1300 nm or 1590 nm lasers available from LaserMax Inc., Rochester, NY. If
30 the heating element 583 is an infrared light source, the materia that
intervenes between the heating element 583 and the fluid chamber 583
preferably transmits at least about 50%, more preferably 80%, of the infrared

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light from the heating element 583.

Figure 13B shows a comparable version of a cut-away view
of eleventh alpha distribution channel 500A11 where the expansion valve
580 is positioned differently.

5   Figure 13C shows a cut-away of a preferred embodiment
where eleventh alpha distribution channel has a first expansion valve 580A, a
second expansion valve 580B and a third expansion valve 580C. These three
valves can be operated sequentially to create a pumping force that moves
liquid into the reaction cell 350B1. For instance, at time one, eleventh

10   distribution channel 500A11 is filled with a liquid and first expansion valve
580A is expanded. At time two, first expansion valve 580A remains
expanded and second expansion valve 580C begins to expand, pushing liquid
into the reaction cell 350B1. At time three, second expansion valve 580B
remains expanded and first expansion valve 580A begins to contract drawing

15   liquid from second alpha feeder channel 570A2 to fill the volume formerly
occupied by the expanded valve. Also at time three, third expansion valve
580C begins to expand, forcing liquid to flow into reaction cell 350B1. At
time four, third expansion valve 580C remains expanded and second
expansion valve 580B begins to contract at the about the same time first

20   expansion valve 580A begins to expand. At time five, first expansion valve
580A is expanded, while the other two expansion valves, 580B and 580C,
are contracted, setting the stage for a new pumping cycle.

E. Electrode-based Liquid Distribution System

Figure 14 shows a top view of a liquid distribution system of
25   the electrode-based liquid distribution system showing how channels from a
number of feeder channels, e.g., alpha through delta feeder channels 670A
through 670D, respectively, can connect to a fifth vertical channel 628 that
connects to a given reaction cell 350.

In Figure 14, alpha feeder channel 670A, beta feeder channel
30   670B, gamma feeder channel 670C and delta feeder channel 670D connect
to alpha first distribution channel 622A, alpha second distribution channel
624A, beta second distribution channel 624B, and beta first distribution

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channel 622B, respectively. Alpha first distribution channel 622A, alpha
second distribution channel 624A, beta second distribution channel 624B,
and beta first distribution channel 622B have an alpha first electrode-based
pump 660A, a beta first electrode-based pump 660B, a gamma first

5   electrode-based pump 660C and a delta first electrode-based pump 660D,
respectively. The alpha first distribution channel 622A connects to fifth
vertical channel 628 via alpha second connector channel 623A and alpha
third connector channel 625A. The beta first distribution channel 622B
connects to fifth vertical channel 628 via beta second connector channel

10   623B and beta third connector channel 625B. Note that alpha second and
beta second connector channels 623A and 623B are in a lower plane than
their connected alpha first distribution channel 622A and beta first
distribution channel 622B, respectively. Alpha second distribution channel
624A and beta second distribution channel 624B each connect directly with

15   fifth vertical channel 628.

Note that the "N" portions (indicated in the figure) of alpha,
beta, gamma and delta feeder channels 670A-670D, respectively, are referred
to as the "feeder channel inlets", while the "S" portions are referred to as the
"feeder channel outlets". The inlets of alpha, beta, gamma and delta feeder

20   channels 670A-670D, have an alpha second electrode-based pump 667A, a
beta second electrode-based pump 667B, a gamma second electrode-based
pump 667C and a delta second electrode-based pump 667D, respectively.
The outlets of alpha, beta, gamma and delta feeder channels 670A-670D,
have an alpha third electrode-based pump 668A, a beta third electrode-based

25   pump 668B, a gamma third electrode-based pump 668C and a delta third
electrode-based pump 668D, respectively. The voltages used to operate the
three electrode-based pumps in the channels that form the three-way
junctions (made up of a feeder channel inlet, a feeder channel outlet and a
distribution channel) can be adjusted to either allow flow into the distribution

30   channel or to block flow into the distribution channel. Preferably, at each
three-way junction, each electrode of the first electrode-based pump 660,
second electrode-based pump 667 and third electrode-based pump 668 that

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is nearest the junction is connected to a common voltage source or ground.

In a preferred embodiment, capillary barriers, for instance located at the four junctions (unnumbered) with fifth vertical channel 628 can be used to limit unintended flow into fifth vertical channel 628. In another

5   preferred embodiment, the alpha and beta first distribution channels 622A and 622B and the alpha and beta second distribution channels 624A and 624B have narrower channel widths that their connected feeder channels 670, so that flow through the distribution channels 622 or 624 is less favored than flow through the feeder channels 670, particularly when the first

10 electrode-based pump 660 in the distribution channels are operated to pump against the unwanted flow. Preferably, one or more of the distribution channels (e.g., alpha first distribution channel 622A) have a channel width at the opening with the three-way junction that is about 50% or less of the width the connected feeder channel 670, more preferably the width is about

15 40% or less of the width the connected feeder channel 670.

Features of other distribution systems described in this application can be applied to this embodiment, irrespective of under which subheading they are described.

F. Controller

20   The controller 10 will typically be an electronic processor. However, it can also be a simpler device comprised of timers, switches, solenoids and the like. The important feature of controller 10 is that it directs the activity of the first pumps 360 and second pumps 361 and, optionally, the activity of external pumps 171. A circuit of thin film transistors (not

25 shown) can be formed on the liquid distribution system to provide power to the wells via leads and electrodes, and to connect them with the driving means such as the controller 10, so as to move liquids through the array. Pins can also be formed substrate which are addressable by logic circuits that are connected to the controller 10 for example.

30 G. Reaction Cells and Reaction Cell Plate

Reaction cells 350 are typically depressions formed in the upper layers of a reaction cell plate 320. The drain 355 to a reaction cell 350

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can be open at the bottom of the reaction cell 350, in which case drainage is controlled kinetically and by negative pressure from the connected channels. Alternatively, the drain 355 may be adjacent to the reaction cell 350 and open at a height greater than the height of the floor of the reaction cell 350,

5   as illustrated in Figure 15A. In Figure 15A, drain 355 is connected to reaction cell 350 by sluice 351. In this case, flushing volumes, which are substantial volumes relative to the volume of the reaction cell but minuscule in absolute amount, are passed through the reaction cell 350 to remove all of a given reactant previously directed into the reaction cell 350. In another

10   alternative, the reaction cell 350 is connected to a alpha first horizontal drain channel 356A and beta first horizontal drain channel 356B, having an alpha drain pump 357A and beta drain pump 357B, respectively, which can be an electrode-based pumps, as illustrated in Figure 15B. The alpha and beta first horizontal drain channels 356A and 356B can be connected to alpha vertical

15   drain channel 358A and beta vertical drain channel 358B, which in turn are connected to an alpha and beta second horizontal drain channel 359A and 359B, respectively. Alpha and beta second horizontal drain channel 359A and 359B open into the alpha drain 355A and beta drain 355B. The alpha and beta pumps 357 are operated under the control of the controller 10,

20   which operates the pumps as appropriate to drain the reaction cell 355. Note that this latter drainage structure includes a form of a capillary barrier 370. Where alpha and beta pumps 357A and 357B are electrode-based pumps, one can be operated so that a - flow would drain the reaction cell 350 and the other so that a + flow would drain the reaction cell 350. In this way, no

25   matter what the flow preference of the liquid in the reaction cell 350, one drain mechanism would push fluid out of the reaction cell 350, while the other merely pushed a limited volume of fluid into the reaction cell 350, yielding a net draining effect.

Another way by which the reaction cell 350 can be

30   controllably drained is to use a bottom drain 355 having an outlet channel that has constrictor, such as one of the constrictors described above with reference to the second preferred embodiment.

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Drains are optional, since in some uses the amount of liquid moved into a reaction cell 350 is less than the reaction cell's volume. If drains are absent, however, vents are required. Vents for the reaction cells 350 are appropriate in other contexts.

5   The reaction cell plate can be reversibly bonded to the next higher plate by, for instance, assuring that the two surfaces are smoothly machined and pressing the two plates together. Or, for example, a deformable gasket, such as a teflon, polyethylene or an elastomeric film (such as a natural rubber, ABS rubber, or polyurethane elastomer film) gasket is

10   interposed between the plates. One way to maintain a force adhering the plates against the gasket is to have a number of vacuum holes cut through the bottom plate and the gasket and applying a vacuum at these locations. Generally, the seal should be sufficient so that the pump used to form the vacuum can be shut down after initially forming the vacuum. The gasket is

15   preferably from about 0.0x5 mils to about 1 mil, more preferably from about 0.1 mils to about 0.3 mils in thickness.

Fluid exiting the bottom of the reaction cell plate 320 can, for instance, simply collect in a catch pan or it can diffuse into a porous substrate such a sintered glass, glass wool, or a fabric material. Alternately,

20   a fifth plate 340 is attached to the underside of the reaction cell and has channels that connect the outlets of the reaction cells 350 to individual collection reservoirs from which fluid can be sampled. For instance, the fifth plate 340 is wider than the reaction cell plate 320 and the collection reservoirs are located at the top surface of the fifth plate 340 in the area not

25   covered by the reaction cell plate 320.

Preferably, synthetic processes conducted in the reaction cells 350 of the liquid distribution system will take place on insoluble supports, typically referred to as "beads", such as the styrene-divinylbenzene copolymerizate used by Merrifield when he introduced solid phase peptide

30   synthetic techniques. Merrifield, *J. Am. Chem. Soc.* 85: 2149, 1963. See, also Barany et al., "Recent Advances in Solid-Phase Synthesis," in *Innovation and Perspectives in Solid Phase Synthesis: Peptides, Polypeptides, and*

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Oligonucleotides, Roger Epton, Ed., collected papers of the 2nd International
Symposium, 27-31 August, 1991, Canterbury, England, p. 29. These
supports are typically derivatized to provide a "handle" to which the first
building block of an anticipated product can be reversibly attached. In the

5   peptide synthesis area, suitable supports include a p-alkoyxbenzyl alcohol
resin ("Wang" or PAM resin) available from Bachem Bioscience, Inc., King of
Prussia, PA), substituted 2-chlorotrityl resins available from Advanced
Chemtech, Louisville, KY, and polyethylene glycol grafted poly styrene resins
(PEG-PS resins) are available from PerSeptive Biosystems, Framingham, MA or

10 under the tradename TentaGel, from Rapp Polymere, Germany. Similar solid
phase supports, such as polystyrene beads, are also used in the synthesis of
oligonucleotides by the phosphotriester approach (see Dhristodoulou,
"Oligonucleotide Synthesis: Phosphotriester Approach," in *Protocols for
Oligonucleotide Conjugates*, S. Agrawal, Ed., Humana Press, N.J., 1994), by

15 the phosphoramidite approach (see Beaucage, "Oligodeoxynucleotide
Synthesis: Phosphoramidite Approach," in *Protocols for Oligonucleotide
Conjugates*, S. Agrawal, Ed., Humana Press, N.J., 1994), by the
H-phosponate approach (see Froehler, Oligodeoxynucleotide Synthesis:
H-Posponate Approach," in *Protocols for Oligonucleotide Conjugates*, S.

20 Agrawal, Ed., Humana Press, N.J., 1994), or by the silyl-phosphoramidite
method (see Damha and Ogilvie, Oligodeoxynucleotide Synthesis:
"Silyl-Phosphoramidite Method," in *Protocols for Oligonucleotide Conjugates*,
S. Agrawal, Ed., Humana Press, N.J., 1994). Suitable supports for
oligonucleotide synthesis include the controlled pore glass (cpg) and

25 polystyrene supports available from Applied Biosystems, Foster City, CA.
Solid supports are also used in other small molecule and polymeric organic
syntheses, as illustrated in oligocarbamate synthesis for organic polymeric
diversity as described by Gorden et al., *J. Medicinal Chem.* 37: 1385-1401,
1994.

30   Preferably, the reaction cells 350 are rectangular with
horizontal dimensions of about 400 microns to about 1200 microns, more
preferably about 500 microns to about 1000 microns, yet more preferably

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about 640 microns, and a depth of about 200 microns to about 400 microns.
Where beads will be used in the reaction cells 350, the depth of the reaction
cells 350 is preferably at least about 50 microns greater than the swelled
diameter of the beads. The support beads typically used as in solid-phase
5   syntheses typically have diameters between about 50 microns and about 250
microns, and reactive site capacities of between about 0.1 mmoles/g and
about 1.6 mmoles/g. Typically, between about 1 and about 10 of such beads
are loaded into a reaction cell 350 to provide a desired capacity of between
about 1 nmole and about 10 nmole per reaction cell 350. Recently, beads
10   have become available that have a diameter that ranges between about 200
microns and about 400 microns, depending on the solvent used to swell the
beads and the variation in size between the individual beads, and a reactive
site capacity of between about 5 nmole and about 20 nmole per bead have
become available. These large beads include the beads sold by Polymer
15   Laboratories, Amhearst, MA. Desirable reactive site functionalities include
halogen, alcohol, amine and carboxylic acid groups. With these large beads,
preferably only one bead is loaded into each reaction cell 350.

        Another option for creating a solid support is to directly
derivatize the bottom of the reaction cell 350 so that it can be reversibly
20   coupled to the first building block of the compound sought to be synthesized.
The chemistry used to do this can be the same or similar to that used to
derivatize controlled pore glass (cpg) beads and polymer beads. Typically, the
first step in this process is to create hydroxyl groups (if they do not already
exist on the support) or amino groups on the support. If hydroxyl groups
25   exist or are created, they are typically converted to amino groups, for
instance by reacting them with gamma-aminopropyl triethoxy silane. Flexible
tethers can be added to the amino groups with cyclic acid anhydrides,
reactions with polymerized alkylene oxides and other methods known to the
art. Examples of such methods are described in Fields et al., "Synthetic
30   Peptides: A User's Guide," W.H. Freeman and Co., Salt Lake City, UT, 1991.
        Methods of creating reactive sites include, for the case
where the reaction cell plate 320 is made of plastic, exposing the bottom of

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the reaction cells 350 to a reactive plasma, such as that created by a
glow-discharge in the presence of ammonia or water, to create NH_2 groups.
Such procedures are described in "Modification of Polymers," Carraher and
Tsuda, eds., American Chem. Soc., Washington, DC, 1980. Another

5   method, useful with glass, ceramic or polymeric substrates, is depositing a
film of silicon monoxide by vapor deposition at low temperature to create
hydroxyl functionalities. Glass surfaces can be treated with alkali, for
instance with KOH or NaOH solutions in water or water/alcohol mixtures, to
expose hydroxyl functional groups. Non-annealed borosilicate glass surfaces,

10   including coatings of non-annealed borosilicate glass created by chemical
vapor deposition, can be etched, for instance with hydrofluoric acid dissolved
in water, to dissolve the regions that are rich in boron, which process creates
a porous structure with a large surface area. This porous structure can be
treated with alkali to expose hydroxyl groups. The degree of reactive site

15   substitution on such surfaces is preferably at least about 83 nmoles per cm^2,
more preferably at least about 124 nmoles per cm^2 (implying a substitution in
500 micron by 500 micron reaction cell 350 of at least about 0.31 nmole),
yet more preferably at least about 256 nmoles per cm^2.

The above described methods for using the bottom of the
20   reaction cells 350 as a solid support can be supplemented by methods that
increase the surface area of the bottom of the reaction cells 350. One
method is to create columnar structures of silicon monoxide, for instance by
thermal evaporation of SiO_x. Another such method is to insert into the
reaction cells fabrics, such as non-woven glass or plastic (preferably fiberglass

25   or polypropylene fiber) fabrics and plasma treating the fabric to create
reactive sites.

Another method uses spin-on glass, which creates a thin film
of nearly stoichiometric SiO_2 from a sil-sesquioxane ladder polymer structure
by thermal oxidation. Sol-gel processing creates thin films of glass-like

30   composition from organometallic starting materials by first forming a
polymeric organometallic structure in mixed alcohol plus water and then
careful drying and baking. When the sol-gel system is dried above the critical

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temperature and pr ssure of the solution, an aerogel results. Aerogels have chemical compositions that are similar to glasses (e.g. SiO_2) but have extremely porous microstructures. Their densities are comparably low, in some cases having only about one to about three percent solid composition,

5   the balance being air.

H. Capillary barriers

Capillary barriers have been described above with reference to Figures 4A and 4B. However, more complex design considerations than were discussed above can, in some cases, affect the design of the capillary

10   barrier. In some cases it is desirable to narrow the sluice formed by second opening 362 or third opening 363 to increase the impedance to flow (i.e., the frictional resistance to flow) as appropriate to arrive at an appropriate flow rate when the associated first pump 360 or second pump 361 is activated. Such a narrowing is illustrated by comparing the sluice of Figure 16A with the

15   narrowed sluice of Figure 16D. The problem that this design alteration can create is that narrower channels can increase capillary forces, thereby limiting the effectiveness of channel breaks.

Thus, in one preferred embodiment, a channel break further includes one or more upwardly oriented sharp edges 369, as illustrated in

20   Figures 16B and 16C. More preferably, a channel break includes two or more upwardly oriented sharp edges 369. In Figure 16B, portion 362A of opening 362 is cut more deeply into first plate 300 to create an open space useful for the operation of upwardly oriented sharp edges 369.

I. Fabrication of Plates, Channels, Reservoirs and Reaction Cells

25   The liquid distribution systems of the invention can be constructed a support material that is, or can be made, resistant to the chemicals sought to be used in the chemical processes to be conducted in the device. For all of the above-described embodiments, the preferred support material will be one that has shown itself susceptible to microfabrication

30   methods that can form channels having cross-sectional dimensions between about 50 microns and about 250 microns, such as glass, fused silica, quartz, silicon wafer or suitable plastics. Glass, quartz, silicon and plastic support

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materials are preferably surface treated with a suitable treatment reagent such as a siliconizing agent, which minimize the reactive sites on the material, including reactive sites that bind to biological molecules such as proteins or nucleic acids. As discussed earlier, the expansion valve liquid distribution

5   system is preferably constructed of a plastic. In embodiments that require relatively densely packed electrical devices, a non-conducting support material, such as a suitable glass, is preferred. Corning borosilicate glass, and Corning 7740 borosilicate glass, available from Corning Glass Co., Corning, NY, are among the preferred glasses.

10   The liquid distribution system of the invention is preferably constructed from separate plates of materials on which channels, reservoirs and reaction cells are formed, and these plates are later joined to form the liquid distribution system. This aspect of the invention is described in some detail with respect to the hydrologic liquid distribution system. Preferably,

15   the reaction cell plate, e.g. reaction cell plate 320, is the bottom plate and is reversibly joined to the next plate in the stack. The other plates forming the distribution system, which preferably comprise two to three plates are preferably permanently joined. This joinder can be done, for instance, using adhesives, or techniques such as glass-glass thermal bonding.

20   One preferred method of permanently joining the plates is to first coat the plate with a layer of glass glaze generally having a thickness between about 50 microns and about 500 microns, more preferably between about 75 microns and about 125 microns. The above thicknesses contemplate that substantial amounts of channel structure will be formed in

25   the glaze layer. Otherwise, the glaze generally has a thickness between about 1 microns and about 100 microns, more preferably between about 10 microns and about 25 microns. These methods are preferably applied to join glass plates. Suitable glazes are available from Ferro Corp., Cincinnati, OH. The glazed plate is treated to create channels, reservoirs, or reaction cells as

30   described below. The glazed plate is positioned against another plate, which preferably is not glazed, and the two plates are heated to a temperature of about the softening temperature of the glaze or higher, but less than the

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softening temperature for the non-glaze portion of the plates.

Another preferred method of permanently joining glass plates uses a field assisted thermal bonding process. It has now been discovered that glass-glass sealing using field assist thermal bonding is possible despite

5   the low conductivity of glass if a field assist bonding material is interposed between the plates to be bonded.

To the top or bottom surface of one glass plate a layer of a field assist bonding material is applied. Preferably, the field assist bonding material layer has a thickness between about 50 nm and about 1,000 nm,

10   more preferably, between about 150 nm and about 500 nm. The field assist bonding material can be a material capable of bonding glass substrates using the method described herein. Preferably, the field assist bonding material is silicon or silica. More preferably, the field assist bonding material is silicon.

The field assist bonding material can be applied to a plate, for

15   instance, by chemical vapor deposition or by a sputtering process where surface molecules are emitted from a cathode when the cathode is bombarded with positive ions from a rare gas discharge and the surface molecules collide with and bond to a nearby substrate. Pursuant to the present invention, silicon layers of between about 150 nm and about 500 nm

20   thickness have been bonded to glass plates under conditions that can be expected to generate an outer surface layer of silicon dioxide, such as an. about 20Å layer, although the sealing process is believed to be effective in the absence of this layer. The coated plate is treated, as needed, to create channels, reservoirs, or reaction cells using the method described below.

25   Alternatively, the plate was so treated prior to coating with the field-assist bonding material. The coated plate is then positioned against another plate, which preferably is not coated, and placed in a field assisted bonding device 700 such as that illustrated in Figure 17. The field assisted bonding device 700 has a heating device 710, such as a heating plate. The field assisted

30   bonding device 700 further has an electrode 720 and a ground 730 that allows a voltage to be applied across the first plate 740 and the second plate 750, to which has been applied a layer of silicon 760. Arrows 770 indicate

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the electric field orientation. Generally, the field assisted bonding is
conducted under a normal atmosphere.

The plates are brought to a temperature that is effective,
when an appropriate electric field is applied across the plates, to accelerate

5   the bonding process. While not wishing to be bound by theory, it is believed
that the combination of a cathode applied to the first glass plate 740 and the
greater exchange-site mobility of ions (such as sodium ions) caused by the
elevated temperature causes an ion depletion on the face of the first glass
plate 740 opposite that to which the cathode is applied. The ion depletion, it

10   is believed, causes a surface charge at the bottom surface of first glass
substrate 740, which correlates with the creation of a strong localized
electrostatic attraction for the second substrate 750. It is clear that this
process creates strong bonding between the substrates and, it is believed that
this is due to the formation of chemical bonds between the silica of the first

15   glass substrate 740 and the silicon coated onto the second glass substrate
750. Preferably, the temperature is brought to from about 200°C to about
600 °C, more preferably from about 300°C to about 450°C. During the
process an voltage typically from about 200 V to about 2,500 V, preferably
from about 500 V to about 1500 V, is applied across the first glass plate 740

20   and second glass plate 750. The voltage most suitably applied varies with
the thickness of the glass plates. The voltage pulls the first glass plate 740
and second glass plate 750, including the silicon layer 760 applied to one of
the plates, into intimate contact. Typically, hermetic sealing is achieved
within minutes to about an hour, depending on the planar dimensions of the

25   glass plates. The time required to achieve adequate sealing varies with,
among other things, the smoothness of the plates, the electrical field
strength, the temperature, and the dimensions of the plates. Bonding
between the plates is typically apparent visually, since it is accompanied by
the disappearance of the interface between the plates and the formation of

30   gray color at the bonded regions that can be seen when an observer looks
through the thinner dimensions of the two plates.

The method described above can be used to bond a glass

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substrate to another glass substrate and to a third glass substrate
simultaneously.

Those of ordinary skill will recognize that while a hot plate is
illustrated as providing the heating for the thermal assisted bonding, other
5   heating devices, including ovens, may be used. It will also be realized that it is
desirable to match, when possible, the coefficients of thermal expansion of
the substrates to be bonded.

The reservoirs, reaction cells, horizontal channels and other
structures of the fluid distribution system can be made by the following
10   procedure. A plate, that will for instance make up one of feedthrough plate
300, distribution plate 310, reaction cell plate 320 or intermediate plate 330,
is coated sequentially on both sides with, first, a thin chromium layer of about
500Å thickness and, second, a gold film about 2000 angstroms thick in
known manner, as by evaporation or sputtering, to protect the plate from
15   subsequent etchants. A two micron layer of a photoresist, such as Dynakem
EPA of Hoechst-Celanese Corp., Bridgewater, NJ, is spun on and the
photoresist is exposed, either using a mask or using square or rectangular
images, suitably using the MRS 4500 panel stepper available from MRS
Technology, Inc., Acton, MA. After development to form openings in the
20   resist layer, and baking the resist to remove the solvent, the gold layer in the
openings is etched away using a standard etch of 4 grams of potassium
iodide and 1 gram of iodine (I_2) in 25 ml of water. The underlying chromium
layer is then separately etched using an acid chromium etch, such as KTI
Chrome Etch of KTI Chemicals, Inc., Sunnyvale, CA. The plate is then etched
25   in an ultrasonic bath of $HF-HNO_3-H_2O$ in a ratio by volume of 14:20:66. The
use of this etchant in an ultrasonic bath produces vertical sidewalls for the
various structures. Etching is continued until the desired etch depth is
obtained. Vertical channels are typically formed by laser ablation.

The various horizontal channels of the distribution system
30   embodiments typically have depths of about 50 microns to about 250
microns, preferably from about 50 microns to about 100 microns, more
preferably from about 50 microns to about 80 microns. The widths of the

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horizontal channels and the diameters of the vertical channels are typically from about 50 microns to about 200 microns, preferably from about 100 microns to about 200 microns, more preferably from about 120 microns to about 150 microns.

5   J. Fabrication of Electrode-Based Pumps

In many embodiments, the liquid distribution systems of the invention require the formation of numerous electrodes for pumping fluids through the liquid distribution system. These electrodes are generally fabricated in the top glass plate of the liquid distribution system. Typically 10 each pair of electrodes is closely spaced (e.g. 50 to 250 microns separation). The electrodes are fabricated with diameters of preferably about 25 microns to about 150 microns, more preferably about 50 microns to about 75 microns. In preferred embodiments, the liquid distribution system has 10,000 reaction cell 350 with each reaction cell 350 having 6 - 10 associated 15 electrode-based pumps. Thus, a liquid distribution system can require about 200,000 to about 300,000 electrodes. To produce such structures using mass production techniques requires forming the electrodes in a parallel, rather than sequential fashion. A preferred method of forming the electrodes involves forming the holes in the plate (e.g., feedthrough plate 300) through 20 which the electrodes will protrude, filling the holes with a metallic thick film ink (i.e., a so-called "via ink", which is a fluid material that sinters at a given temperature to form a mass that, upon cooling below the sintering temperature, is an electrically conductive solid) and then firing the plate and ink fill to convert the ink into a good conductor that also seals the holes 25 against fluid leakage. The method also creates portions of the electrodes that protrude through the plate to, on one side, provide the electrodes that will protrude into the liquids in fluid channels and, on the other side, provide contact points for attaching electrical controls.

For example, holes are drilled in 500 micron thick plates of 30 borosilicate glass using an excimer laser. Holes having diameters between 50 and 150 microns are then filled with thick film inks, using an commercial Injection Via-fill Machine (Pacific Trinetics Model #VF-1000, San Marcos,

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CA). It has been unexpectedly discovered that only select formulations of via
inks sufficiently function to fill such high aspect ratio holes such that the fired
ink adheres to the sides of the holes, does not crack during the firing process,
and seals the holes against fluid flow. One parameter that is important to so
5   forming sealed, conductive conduits through high aspect holes is selecting
metal powder and glass powder components for the via ink that have
sufficiently fine dimensions. One suitable formulation uses: 12-507 Au
powder (Technic. Inc., Woonsocket, RI), 89.3 % w/w; F-92 glass (O.
Hommel Co., Carnegie, PA), 5.7 % w/w; 15 % w/v ethyl cellulose N-300
10   (N-300, Aqualon, Wilmington, DE) in Texanol™ (monoisobutarate ester of
2,2,4-trimethyl-1,3-pentandiol, Eastman Chemical Products, Kingsport, TN),
2.4% w/w; 15 % w/v Elvacite 2045™ (polyisobutyl methacrylate) in
Terpineol T-318 (mixed tertiary terpene alcohols, Hercules Inc., Wilmington,
DE), 2.1 % w/w; and Duomeen TDO™ (N-tallow alkyl trimethylenediamine
15   oleates, Akzo Chemicals, Chicago, IL), 0.5 % w/w. The gold powder from
Technic, Inc. has an average particle diameter of 0.9 microns. Another
suitable formulation uses: Ag Powder Q powder (Metz, South Plainfield, NJ),
80.8 % w/w; F-92 glass (O. Hommel Co. Carnegie, PA), 5.2 % w/w; VC-1
resin (37% w/w Terpineol T-318, 55.5% w/w butyl carbitol, 7.5% w/w
20   ethylcellulose N-300, Aqualon, Wilmington, DE), 3.7 % w/w; 15 % w/v
ethyl cellulose N-300 in Texanol™, 4.0% w/w; 15 % w/v Elvacite 2045™
(polyisobutyl methacrylate) in Terpineol T-318, 4.1 % w/w; Duomeen
TDO™, 0.6 % w/w; and Terpineol, 1.6 % w/w. These formulations were
fired at 550°C to form high aspect ratio conductive conduits.
25   When the size of the glass or metal powders increases, good
filling properties (lack of cracking, good sealing against liquids, good
adherence to sides of hole) can often still be obtained by decreasing the
amount of organic in the via ink.
                 The devices used to insert via inks into holes in a plate
30 typically include a metal stencil with openings corresponding to the openings
in the plate. Via ink is applied above the stencil, which rests on the plate,
and a bladder device is used to pressurize the ink to force it to fill the holes.

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After filling, the plate with its via ink-filled holes is removed for further processing, as described below.

Prior to firing, much of the organic component is evaporated away by, for example, placing the ink-filled plate in a oven (e.g. at 100 °C)
5   for one to five minutes. Preferably, the firing is conducted at a temperature from about 450°C to about 700°C, more preferably from about 500°C to about 550°C. However, the upper end of the appropriate firing temperature range is primarily dictated by the temperature at which the plate being treated would begin to warp. Accordingly, with some types of plates much higher
10   temperatures could be contemplated.

To assure that there is conductive material that protrudes above and below the glass plate after firing, the top and bottom surface of the plate can be coated with a sacrificial layer of thicknesses equaling the length of the desired protrusions. The sacrificial layers can be applied before
15   or after the holes are formed in the plate. If before, then the holes are formed through both the glass plate and the sacrificial layers. If after, then (a) corresponding openings through the sacrificial layers can be created by creating a gas pressure difference from one side of the plate to the other, which pressure difference blows clear the sacrificial material covering the
20   holes or (b) such openings through at least the top sacrificial layer are created when the pressure of the ink pushes through the sacrificial layer and into the holes (leaving an innocuous amount of sacrificial layer material in the holes). An appropriate sacrificial layer burns away during the firing process. Sacrificial layers can be made coating a plate with, for instance, 5 - 25 w/w
25   % mixtures of ethyl cellulose resin (e.g., Ethyl Cellulose N-300, Aqualon, Wilmington, DE) dissolved in Terpineol T-318™ or Texanol™, or 5 - 50 % w/w mixtures of Elvacite 2045™ in Terpineol T-318™. After firing, the surfaces of the electrode can be enhanced plating metals, such as nickel, silver, gold, platinum, rhodium, etc. The depositions can be performed using
30   standard electrolytic and/or electroless plating baths and techniques.

Preferably, where a plate that is to contain etched openings will be processed to include electrodes, the etching occurs first, followed by

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coating with the sacrificial layer and forming the electrode holes.

In an alternate method of manufacture, for each pump, two or more metal wires, for example gold or platinum wires about 1-10 mils in diameter, are inserted into the openings in the channel walls about, e.g., 150
5   microns apart. The wires were sealed into the channels by means of a conventional gold or platinum via fill ink made of finely divided metal particles in a glass matrix. After applying the via fill ink about the base of the wire on the outside of the opening, the channel is heated to a temperature above the flow temperature of the via fill ink glass, providing an excellent seal between
10 the wires and the channel. The via ink, which is used to seal the holes, can be substituted with, for instance, solder or an adhesive.

I. Miscellaneous Features

In the case where the temperature of a particular well is to be monitored or changed, a means of heating or cooling the well is built into
15 the well, as will be further explained below with reference to Fig. 20. The first well 36 in this example has deposited on its bottom surface a thin film 57 of a suitable metal oxide, such as tin oxide or indium tin oxide. The thin film 57 is connected by means of an electrically conductive metal connection 58 to the end or outer edge of the well 36. The tin oxide coating 57 serves as
20 a heater element for the well 36. The sides of the well 36 have a surface bimetal film 59 and leads 60, suitably made of chromel-alumel alloys, forming a thermocouple to measure the temperature in the well when a source of current is applied to the tin oxide coating 57 and to the leads 58. A voltage applied to the well 36 via electrodes 56 deposited on the backside as shown
25 regulates the temperature in the well. The amount of current applied can be regulated by the controller 10 in response to the temperature measured through the leads 60.

In some applications of the liquid distribution system a significant vapor pressure may develop in reaction cell 350, causing a back
30 pressure into the distribution plate 310. Thus preformed valves 70 (see Figure 21A) formed of bimetallic materials as described by Jerman et al, "Understanding Microvalve Technology", Sensors, September 1994 pp 26-36

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can be situated in third vertical channel 390. These materials have a thermal

expansion mismatch. When the temperature in the reaction cell 350 is low,

the ball valve 62 is in its normal position permitting free flow of fluids into the

well 36 (see Figure 21A). As the temperature in the well 36 increases, the

5   ball valve 62 moves to a cooler position (Figure 21B) blocking the third

vertical channel 390 to isolate the reaction cell 350, thereby preventing fluids

from passing into and out of the first well 36. Alternatively, a conventional

check valve having a bearing, such as a bearing made of quartz or

polytetrafluoroethylene polymer can be used to isolate the reaction cell 350.

10   Where it is important to have the capability to have fluid flow counter to the

direction established by the check valve, the check valve can have an

insulating or magnetic bearing, which can be moved to allow such counter-

flow with externally applied electrostatic-or magnetic fields.

15   EXAMPLES

Example 1 - Liquids pumped with a simple electrode-based pump

        Using the 1 mm capillary with a two electrode-pump

described above in Section B.ii., a number liquids have been tested, including

the following solvents:

Solvent	Flow direction	voltage applied
N-methyl-pyrrolidinone (NMP)	+	1470
Dimethyl formamide (DMF)	+	390
Dichloromethane (DCM)	-	686
Methanol (MeOH)	-	489
Isopropanol (IPA)	+	
Acetone	+	
Acetonitrile	+	

30

The following solutions in NMP, at 0.1M unless otherwise indicated, have

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been tested:

Reagent	Flow direction
trans-4-(trifluoromethyl)-cinnamic acid	-
5-benzimidazolecarboxylic acid	-
N,N-dicyclohexylcarbodiimide	+
isobutylamine	+
2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)	No flow at 0.1M, flow occurs lower concentrations (0.01 - 0.1M)

The following solutions in DMF, all at 0.1M excepting piperidine, which was 20% v/v, have been tested:

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Reag nt	Flow direction
p-carboxybenzenesulfonamide	- P
4-fluorophenylacetic acid	- P
4-methoxyphenylacetic acid	- P
m-trifluoromethylbenzoic acid	- P
3-(4-methoxyphenyl)propionic acid	-
4-bromocinnamic acid	- P
terephthalic acid	- P
isophthalic acid	- P
1,3-phenylenediacetic acid	- P
1,4-phenylenediacetic acid	- P
3-(4-carboxyphenyl) propionic acid	- P
1,4-phenylenedipropionic acid	- P
4,4'-oxybis (benzoic acid)	- P
4,4'-dicarboxybenzophenone	- P
piperidine	+
1,3-diisopropylcarbodiimide	+
allylamine	+
butylamine	+
isoamylamine	+
propylamine	+
isobutylamine	+
cyclohexylamine	+
heptylamine	+
benzylamine	+
phenylamine	+ P
3-amino-1-propanol	+ P
2-aminoethanol	+

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Reagent	Flow direction˙
4-(aminomethyl) pyridine	+ P
4-(2-aminoethyl) morpholine	+ P
1-(3-aminopropyl) imidazole	+
triphenylphosphine	+
4-(aminopropyl) morpholine	+
9-fluorenemethanol	+
p-nitrobenzyl alcohol	+
p-(methylthio) benzyl alcohol	-
o-aminobenzyl alcohol	+
2-methoxybenzyl alcohol	+
2-(triflouromethyl) benzyl alcohol	+
2-amino-3-phenyl-1-propanol	+ P
diethylazodicarboxylate	- P
4-dimethylaminopyridine	+ P
carbazole	+
azobenzene	+
3,4-dihydroxybenzoic acid	- P
4-methylmorpholine N-oxide	+
3-cyanobenzoic acid	No flow
4-nitrophenylacetic acid	No flow, at 0.1M, flow occurs lower concentrations (0.01 - 0.1M)
2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)	No flow, at 0.1M, flow occurs lower concentrations (0.01 - 0.1M)
2,3-dichloro-5,6-dicyano-1,4-benzoquinone	+ weak
tetrapropylammonium perruthenate	No flow

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Reagent	Flow direction[*]
I-oxo-2,2,6,6-tetramethylpiperdinium chloride	No flow
5-benzimidazolecarboxylic acid	N.D.[δ]
4-(aminomethyl) benzoic acid	N.D.
4-(aminomethyl) benzoic acid	N.D.
N,N-diisopropylethylamine	N.D.
isobuylamine	N.D.
glutathione (SH)	N.D.

[*] Those directional indicators (" + " or "-") followed by a "P" indicate that flow was achieved using a pulsed voltage program pursuant to Figure 8, where $r_1 = 0.1 - 1$ ms and $r_2 = 3.0 - 10$ ms.

[δ] "N.D.", in this table and the tables below, indicates either that the solute was immiscible with the solvent or that visual inspection suggested that it had decomposed.

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The following solutions in DCM, at 0.1M unless otherwise indicated , have
been tested:

Reagent	Flow direction
allylamine	-
butylamine	-
cyclohexylamine	-
1-(3-aminopropyl) imidazole	-
diethylazodiacarboxylate	-
TP Palladium	-
isobutylamine	-
isoamylamine	
propylamine	-
1-(3-aminopropyl)imidazole	-
p-carboxybenzenesulfonamide	N.D.
2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)	N.D.

Those directional indicators (" + " or "-") followed by a "P"
indicate that flow was achieved using a pulsed voltage
program pursuant to Figure 8, where $r_1 = 0.1$ - 1 ms and
$r_2 = 3.0$ - 10 ms.

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The following solutions in methanol, all at 0.1M, have been tested:

Reagent	Flow direction*
4-fluorophenylacetic acid	-
9-fluorenemethanol	- P
p-(methylthio) benzyl alcohol	-
(R) sec-phenethyl alcohol	-
3-cyanobenzoic acid	No flow
4-nitrophenylacetic acid	- weak
allylamine	No flow
2-aminoethanol	No flow
2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)	N.D.
isobutylamine	N.D.
isomylamine	N.D.

Those directional indicators (" + " or "-") followed by a "P"
indicate that flow was achieved using a pulsed voltage
program pursuant to Figure 8, where $r_1 = 0.1 - 1$ ms and
$r_2 = 3.0 - 10$ ms.

Example 2 - An electrode-pump based preferential flow system

A channel system was fabricated on two inch by two inch by
20 mil plates of 211 Corning glass (Corning Glass Co., Corning, NY) to
confirm that liquids can be switched to a desired flow pathway by controlling
the voltages applied to certain electrode-based pumps. As illustrated in
Figures 18A and 18B, first channel 804 (2,600 μm long by 150 μm wide by
100 μm deep), second channel 805 (550 μm long by 100 μm wide by 100
μm deep), third channel 806 (800 μm long by 275 μm wide by 100 μm deep),
fourth channel 807 (200 μm long by 100 μm wide by 100 μm deep), fifth
channel 808 (550 μm long by 100 μm wide by 100 μm deep) and sixth
channel 809 (2,500 μm long by 150 μm wide by 100 μm deep) were
fabricated on channel plate 810 (not shown). Also fabricated on the channel

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plate 810 were first well 800A, second well 800B and third well 800C, which
were connected by the channels. An electrode plate 820 was overlaid and
sealed to the channel plate 810 by field assisted thermal bonding. The
electrode plate 820 had openings into first well 800A and second well 800B

5   (not illustrated). Third well 800C included a center drain 855. The electrode
plate 820 further had platinum electrodes, fabricated by inserting 25 μm
wires. The electrodes included first platinum electrode 801A, second
platinum electrode 801B, third platinum electrode 801C, fourth platinum
electrode 802A, fifth platinum electrode 802B, third platinum electrode 802C,

10 and the two electrodes comprising gamma electrode-based pump 803. First
platinum electrode 801A, second platinum electrode 801B and third platinum
electrode 801C make up alpha electrode-based pump 801, while fourth
platinum electrode 802A, fifth platinum electrode 802B and sixth electrode
802C make up beta electrode-based pump 802.

15   Figure 18A shows methanol flowing from first well 800A to
second well 800B, while bypassing third well 800C. This is done by applying
160 V to alpha electrode-based pump 801. Figure 18B shows methanol
flowing from second well 800B to third well 800C while bypassing first well
800A. This is done by applying 200 V to beta electrode-based pump 802,

20 100 V to gamma electrode-based pump 803 and 120 V to alpha
electrode-based pump 801, where the polarity at beta and gamma
electrode-based pumps 802 and 803 favored flow into the third well 800C,
and the polarity at alpha electrode-based pump 801 favored flow away from
first well 800A.

25 Example 3 - Electrode-based pumping past capillary barriers
              Figure 19 shows a prototype liquid distribution system
fabricated pursuant to the hydrologic liquid distribution system. The
distribution system was constructed from three plates of Corning 7740 glass,
Corning Glass, Inc., Corning, NY which plates became top plate 910,

30 intermediate plate 920 and bottom plate 930. The top of intermediate plate
920 was coated with silicon as described above. In top plate 910 were
formed, by laser drilling, first hole 901A, second hole 901B, third hole 902A,

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fourth hole 902B, fifth hole 903A, sixth hole 903B, seventh hole 904A and

eighth hole 904B, which holes each had a diameter of 75 μm. First and

second holes 901A and 901B were used to form first model electrode-based

pump 961. Third and fourth holes 902A and 902B were used to form

5   second prototype electrode-based pump 962. Fifth and sixth holes 903A and

903B were used to form third prototype electrode-based pump 963. Seventh

and eighth holes 904A and 904B were used to form fourth model prototype

electrode-based pump 964. The electrodes in each of first through fourth

prototype electrode-based pumps, 961 - 964, were separated by 200 μm .

10   By etching, alpha opening 905, beta opening 906 and gamma opening 907

were formed on the underside of top plate 910. By laser drilling, ninth hole

908 and tenth hole 909, each with a diameter of 150 μm, were formed

through upper plate 910.

In intermediate plate 920 were formed first prototype

15   channel 911 (made up of segments 911A - 911D) and second prototype

channel 912 (made up of segments 912A - 912D). First and second

prototype channels 911 and 912 having a depth of 80 μm and a width of

150 μm . The entries into these two prototype channels 911 and 912 are

provided by ninth hole 908 and tenth hole 909, respectively. First reaction

20   cell access hole 913 and second reaction cell access hole 914, each with a

diameter of 150 μm , were laser drilled through the intermediate plate 920.

In the underside of intermediate plate 920, a delta opening 915 was formed,

which delta opening 915 connects the reaction cell 950 to first and second

prototype drain holes 921 and 922.

25   In the bottom plate 930, the reaction cell 950 was formed by

etching. First prototype drain hole 921 and second prototype drain hole 922

were laser drilled through bottom plate 920. The top plate 910 and

intermediate plate 920 were bonded together by field assisted thermal

bonding.

30   When methanol was introduced into first prototype channel

911, the liquid was stopped from flowing into reaction cell access hole 913

by the capillary barrier formed by the structure at alpha opening 905.

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Correspondingly, the capillary barrier formed by the structure at beta opening 906 prevented methanol flow into the reaction cell access hole 914. Flow into the reaction cell access holes 913 or 914, by either route, could be initiated by activating the appropriate pumps. For instance, to pump

5 methanol through first prototype channel 911, first prototype electrode-based pump 901 and second prototype electrode-based pump 902 were biased by applying 200 V. Flow through the prototype channel 911 was observed.

Example 4 - Combinatorial Synthesis of Oligonucleotide

This synthesis begins with a number of polystyrene

10 beads onto which is synthesized, by the phosphoramidite method, a protected oligonucleotide having a sequence (5' to 3'): GGAGCCATAGGACGAGAG. See, for instance, Caruthers et al., *Methods in Enzymology* 211: 3-20, 1992, for further discussion of oligonucleotide synthetic methods. The functionalized polystyrene beads, available from Bacham Bioscience (King of

15 Prussia, PA) are inserted into each of the reaction cells of a microscale liquid distribution system having 4 X 4 reaction cells. The liquid distribution system has four first reservoirs, reservoir-1, reservoir-2, reservoir-3 and reservoir-4, each of which can address any reaction cell in the 4 X 4 array. The liquid distribution system has four second reservoirs, reservoir-5, reservoir-6,

20 reservoir-7 and reservoir-8, each of which second reservoirs can address the four reaction cells along a given row (i.e., the reaction cells aligned along an EW axis). Further, the liquid distribution system has four third reservoirs, reservoir-9, reservoir-10, reservoir-11 and reservoir-12, each of which third reservoirs can address any of the four reaction cells in the corresponding

25 column (i.e., reaction cells aligned along an NS axis).

The following process steps are executed:

1.   Each of the reaction cells in the distribution system is washed with acetonitrile from reservoir-1.

2.   3% trichloro acetic acid (TCA) in

30 dichloromethane, from reservoir-2, is pumped through all of the reaction cells. This solution is effective to remove the dimethoxytrityl protecting groups at the 5' ends of the oligonucleotides on the beads.

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3.   All of the reaction cells in the liquid distribution system are again flushed with acetonitrile from reservoir-1.

4.   To the four reaction cells connected to reservoir-5, a mixture of 0.1M protected adenine phosphoramidite in acetonitrile is added.

5 This addition is effective to attach protected adenosine groups to the 5' ends of the oligonucleotides in the four reaction cells connected to reservoir-5. To the four reaction cells connected to reservoir-6, a mixture of 0.1M protected cytosine phosphoramidite in acetonitrile is added. This addition is effective to attach protected cytosine groups to the 5' ends of the oligonucleotides in the

10 four reaction cells connected to reservoir-6. To the four reaction cells connected to reservoir-7, a mixture of 0.1M protected guanosine phosphoramidite in acetonitrile is added. This addition is effective to attach protected guanosine groups to the 5' ends of the oligonucleotides in the four reaction cells connected to reservoir-7. To the four reaction cells connected

15 to reservoir-8, a mixture of 0.1M protected thymidine phosphoramidite in acetonitrile is added. This addition is effective to attach protected thymidine groups to the 5' ends of the oligonucleotides in the four reaction cells connected to reservoir-7.

5.   The reaction cells are washed with acetonitrile

20 from reaction cells from reservoir-1.

6.   The reaction cells are flushed with acetic anhydride:2,6-lutidine:tetrahydrofuran 1:1:8 from reservoir-3. This solution is effective to cap any oligonucleotide chains that did not react with the added monomer.

25   7.   The reaction cells are flushed with 1.1 M tetrabutylperoxide in dichloromethane. This step is effective to oxidize the phosphite triester, which links the newly added monomer to the oligonucleotide, to a phosphate triester.

8.   Steps 1-3 are repeated.

30   9.   To the four reaction cells connected to reservoir-9, a mixture of 0.1M protected adenine phosphoramidite in acetonitrile is added. This addition is effective to attach protected adenosine

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groups to the 5' ends of the oligonucleotides in the four reaction cells
connected to reservoir-9. To the four reaction cells connected to
reservoir-10, a mixture of 0.1M protected cytosine phosphoramidite in
acetonitrile is added. This addition is effective to attach protected cytosine

5   groups to the 5' ends of the oligonucleotides in the four reaction cells
connected to reservoir-10. To the four reaction cells connected to
reservoir-11, a mixture of 0.1M protected guanosine phosphoramidite in
acetonitrile is added. This addition is effective to attach protected guanosine
groups to the 5' ends of the oligonucleotides in the four reaction cells

10   connected to reservoir-11. To the four reaction cells connected to
reservoir-12, a mixture of 0.1M protected thymidine phosphoramidite in
acetonitrile is added. This addition is effective to attach protected thymidine
groups to the 5' ends of the oligonucleotides in the four reaction cells
connected to reservoir-12.

15   The above outlined process is effective to generate 16
separate oligonucleotides, each with a distinct dinucleotide sequence at the 5'
end. Similar synthetic methods can be applied to create various combinatorial
molecules, including peptides and other molecules such as those having
potential pharmacological activity or those useful for diagnostic or other

20   analytical application.

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IN THE CLAIMS:

1.   A distribution system comprising an alpha reservoir
and a beta reservoir , a first set of parallel and adjacent first and second
feeder channels and a second set of parallel and adjacent third and fourth
5   feeder channels which are offset from the first and second feeder channels,
wherein (a) the first and third feeder channels are connected to the alpha
reservoir by a first connector channel that is situated above or below the
second and fourth feeder channels and are independent of the beta reservoir
and (b) the second and fourth feeder channels are connected to the beta
10   reservoir by a second connector channel that is situated above or below the
first and third feeder channels and are independent of the alpha reservoir.

2.   The distribution system of claim 1, wherein the first
and second feeder channels are each connected to a first set of two or more
15   reaction cells and the third and fourth feeder channels are each connected to
a second set of two or more reaction cells.

3.   The distribution system of claim 2, wherein the feeder
channels of the first and second feeder channel sets are parallel to each other.
20

4.   A liquid distribution system comprising:
(a)   a distribution plate comprising:
a least two first reservoirs having a common
first fill level;
25   at least one feeder channel connected to and
extending from each of the first reservoirs, each feeder channel having a
horizontal feeder channel segment, wherein the top of the horizontal feeder
channel segment is below the first fill level;
at least one distribution channel connected to
30   each feeder channel and having a second fill level at substantially the same
elevation as the first fill level; and
a first liquid level maintaining means for

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maintaining the liquid level in each first reservoir at the first fill level,

wherein filling each first reservoir to the first fill level results in the filling of the connected feeder channels and filling the connected distribution channels to the second fill level.

5

5.   The distribution system of claim 4, wherein in the distribution plate one feeder channel from each of the at least two first reservoirs are parallel and adjacent to one another.

10   6.   The distribution system of claim 5, wherein each of the parallel feeder channels is connected to a separate distribution channel positioned perpendicular to the horizontal feeder channel segments of the parallel feeder channels, and each of the connected distribution channels are parallel and adjacent to one another.

15

7.   The distribution system of claim 6, comprising in the distribution plate at least four first reservoirs and at least two sets of parallel, adjacent feeder channels, wherein each of the feeder channels in each set is connected to a separate reservoir, and wherein each set of parallel, adjacent
20 feeder channels is connected to at least two sets of adjacent, perpendicular distribution channels.

8.   The distribution system of claim 4, wherein in the distribution plate the horizontal feeder channel segments connected to the
25 first reservoirs are substantially parallel to one another and wherein the distribution plate comprises one or more second reservoirs, each having a third fill level, a second liquid level maintaining means for maintaining the liquid level in each second reservoir at the third fill level, and an second reservoir extension channel the top of which is at substantially the same
30 elevation as the third fill level.

9.   The distribution system of claim 8, wherein the second

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reservoir extension channels are substantially perpendicular to the horizontal
feeder channel segments and one or more of the second reservoir extension
channels each cross above at least one horizontal feeder channel segment.

5   10.   The distribution system of claim 9, further comprising
one or more buffer channels, wherein at least one buffer channel is positioned
adjacent to at least two first distribution channels and at least one second
reservoir extension channel.

10   11.   The distribution system of claim 4, further comprising:

               (b)   a controller; and

               (c)   a feedthrough plate attached to the top of the
distribution plate comprising supply channels extending through the
feedthrough plate to the first reservoirs and electrical leads extending through
15   the feedthrough plate,

                     wherein the distribution plate comprises pumps at
each distribution channel connected to the controller by the electrical leads
extending through the feedthrough plate.

20   11.   The distribution system of claim 11, further
comprising:

               (d)   a reaction well plate having one or more reaction cells
that can be positioned below the distribution plate,

                     wherein the distribution plate comprises conduits by
25   which liquid can be moved from the distribution channels to the reaction cells
under the control of the pumps.

               12.   The distribution system of claim 11, wherein the first
liquid level maintaining means comprises an overflow release channel
30   connected to each first reservoir that drains any liquid above the first fill level.

               13.   The distribution system of claim 11, wherein the

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pumps in the distribution plate each comprise a first electrode and a second electrode and the controller can direct a pulsed voltage to the pumps to cause pumping.

5   14.   The distribution system of claim 13, wherein the controller comprises an electrical data storage device comprising a database of pumping programs for pumping one or more liquids.

           15.   The distribution system of claim 13, wherein one or
10   more of the pumps further comprise a third electrode and, for the three electrode pumps, the controller can (i) direct a pulsed voltage across the first and second electrodes or (ii) across the first and third electrodes.

           16.   A liquid distribution system comprising:
15   (a)   a reservoir,
           (b)   a channel addressable by the reservoir having a
capillary barrier.

           17.   The liquid distribution system of claim 16, wherein the
20   capillary barrier comprises a dam.

           18.   The liquid distribution system of claim 16, wherein the capillary barrier comprises one or more upwardly oriented sharp edges.

25   19.   The liquid distribution system of claim 1, further comprising a pump associated with the channel for pumping liquid past the capillary barrier.

           20.   The liquid distribution system of claim 19, further
30   comprising a reaction well and a conduit from the outlet of the capillary barrier to the reaction well.

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21.   The liquid distribution system of claim 19, wherein the pump is an electrode-based pump.

22.   A liquid distribution system comprising:

5   one or more continuous flow channels, each having a upstream end and a downstream end, wherein the continuous flow channels have an open zone at the downstream end,

for each continuous flow channel, a branch channel extending off of that continuous flow channel in the open zone, and

10   for each continuous flow channel, an alpha constrictor for constricting flow in the open zone located downstream of the branch channel inlet,

wherein when a liquid flows through one of the continuous flow channels from the upstream to the downstream end a first

15   ratio amount of fluid is diverted into the branch channel when the alpha constrictor is not operating and a second ratio amount, which is greater than the first ratio amount, flows into the branch channel when the alpha constrictor is in operation.

20   23.   The distribution system of claim 22, further comprising, for at least one branch channel, a beta constrictor for constricting flow through the branch channel.

24.   The distribution system of claim 22, wherein the

25   system has two or more continuous flow channels and further comprising a buffer channel addressable by at least two continuous flow channels via their associated branch channels.

25.   The distribution system of claim 24, further comprising

30   a reaction cell and wherein the buffer channel is connected to a reaction cell conduit to the reaction cell and to an overflow conduit.

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26.   The distribution system of claim 25, wherein the reaction cell conduit has a gamma constrictor and the overflow conduit has a delta constrictor.

5   27.   A distribution system comprising:
            a reaction cell,
            two or more feeder channels,
            a separate conduit for each feeder channel connecting that feeder channel to the reaction cell, and
10   a expansion valve for each conduit, wherein the expansion valve has an expanded state that fills a cross-section of the conduit and prevents fluid flow through the conduit and an contracted state that allows fluid flow through the conduit.

15   28.   The distribution system of claim 27 further comprising at least about two cells, each separately addressable by two or more feeder channels via conduits each having a bubble expansion valve.

            29.   The distribution system of claim 27, wherein at least
20   one conduit has two or more expansion valves.

            30.   The distribution system of claim 29, wherein at least one conduit has three or more expansion valves which can be operated in concert to pump liquid from the connected feeder channel into the reaction
25   cell.

            31.   A liquid distribution system comprising:
            one or more feeder channels each made up of a feeder channel inlet and a feeder channel outlet and, connected to each such feeder
30   channel, a distribution channel, each feeder channel having a three-way junction connecting a feeder channel inlet, a feeder channel outlet and the connected distribution channel, the distribution channel connecting with a

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reaction cell;

for each such three-way junction, a first electrode-based pump in the feeder channel inlet or in the feeder channel outlet; and

for each such three-way junction, a second electrode-based
5   pump in the distribution channel,

wherein the voltages applied to the first electrode-based pump and the second electrode-based pump can be chosen (a) so that fluid in one of the feeder channels can be moved from the feeder channel inlet to the feeder channel outlet of the feeder channel with a first amount of flow into
10   the connected distribution channel or (b) so that a second amount of flow which is greater than the first amount proceeds via the connected distribution channel.

32.   The distribution system of claim 31, wherein for at
15   least one such three-way junction there is a third electrode-based pump in whichever of the feeder channel inlet or feeder channel outlet lacks the first electrode-based pump.

33.   The distribution system of claim 31, wherein one or
20   more distribution channels have a capillary barrier.

34.   The distribution system of claim 31, wherein the opening of one or more distribution channels at the three-way junction has a width that is about 50% or less of the width of the connected feeder channel.
25

35.   A liquid distribution system suitable for conducting reactions for chemical syntheses or chemical reactions involved in an assay, the distribution system comprising a reaction cell, a channel connected to the reaction cell and a pump situated in the channel comprising a first electrode,
30   second electrode and third electrode.

36.   The distribution system of claim 35, wherein the first

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electrode and second electrode are spaced between about 100 microns and
2500 microns apart, and the spacing between the third electrode and the
nearest of the first or second electrodes is between about 100 microns and
1000 microns.

5

        37.   The distribution system of claim 35, wherein the first
electrode and second electrode are spaced between about 250 microns and
1000 microns apart, and the spacing between the third electrode and the
nearest of the first or second electrodes is between about 250 microns and
10 ᐧ 1000 microns.

        38.   The distribution system of claim 35, further comprising
a controller that controls the operation of the pump, wherein the controller
can operate any pair of electrodes in the pump.

15

        39.   The distribution system of claim 38, wherein the
controller comprises an information storage means which stores pumping
protocols for one or more liquids, each of which stored protocols include a
designation of an appropriate pair of pump electrodes for pumping the liquid
20 of the protocol.

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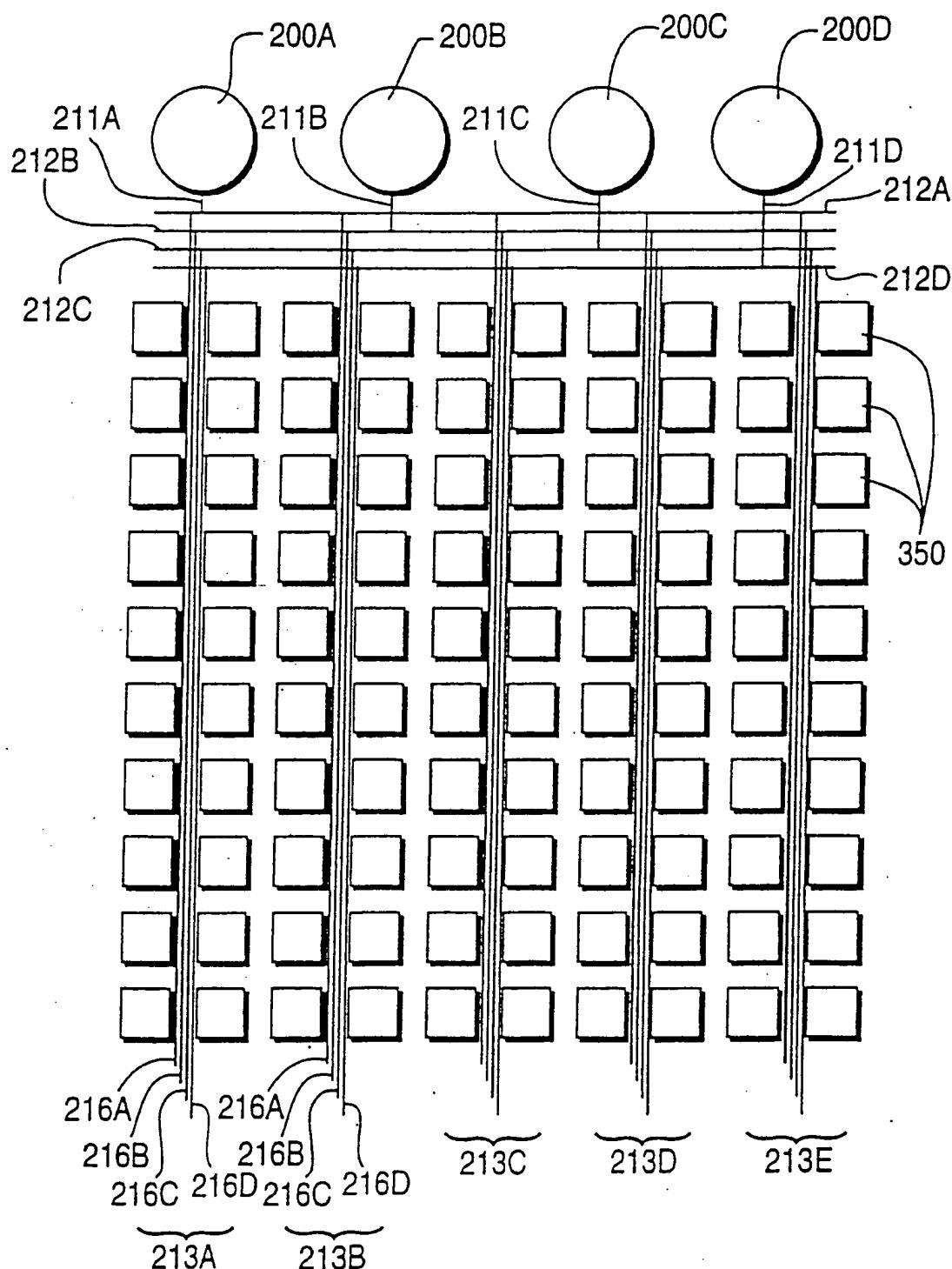
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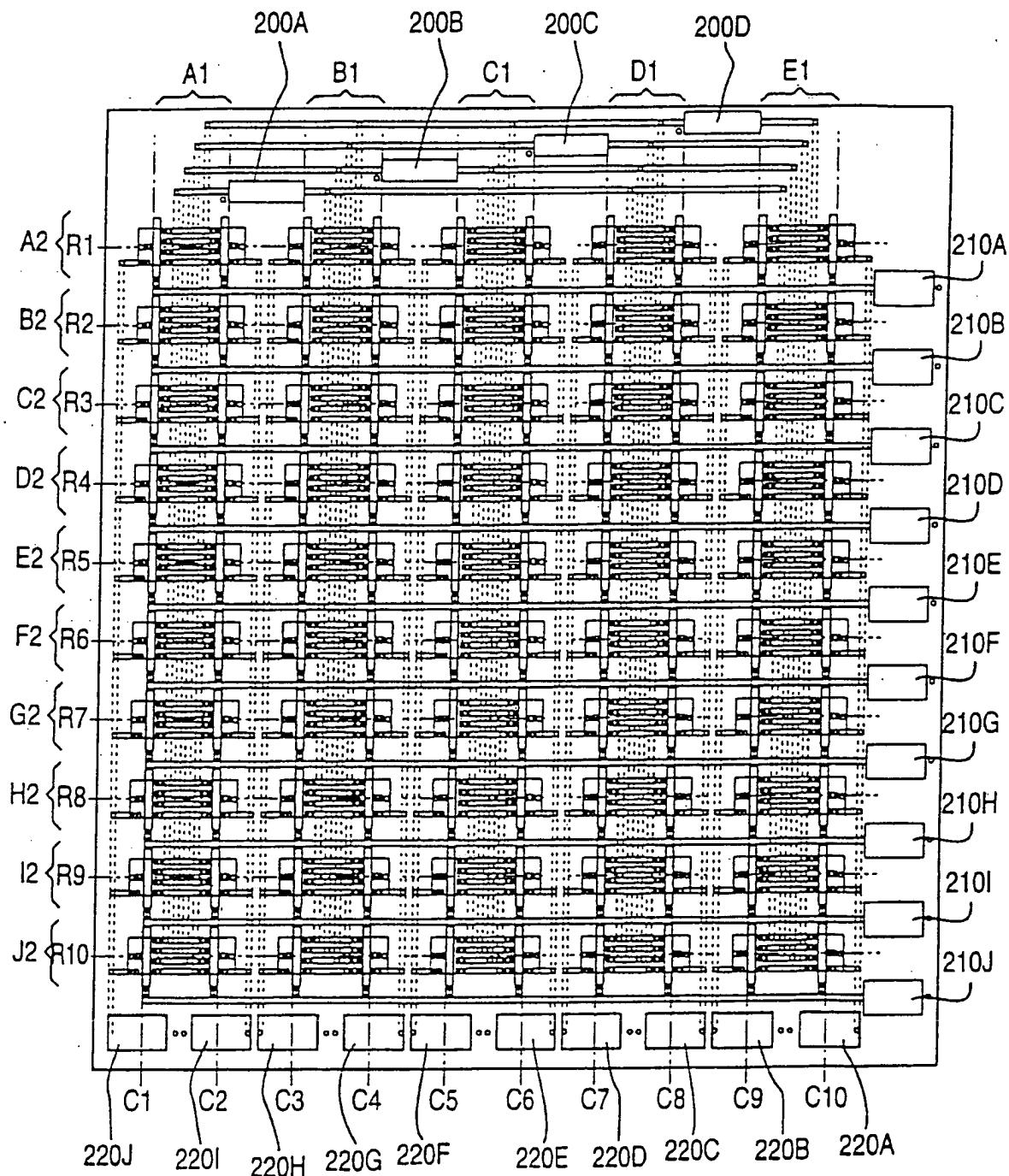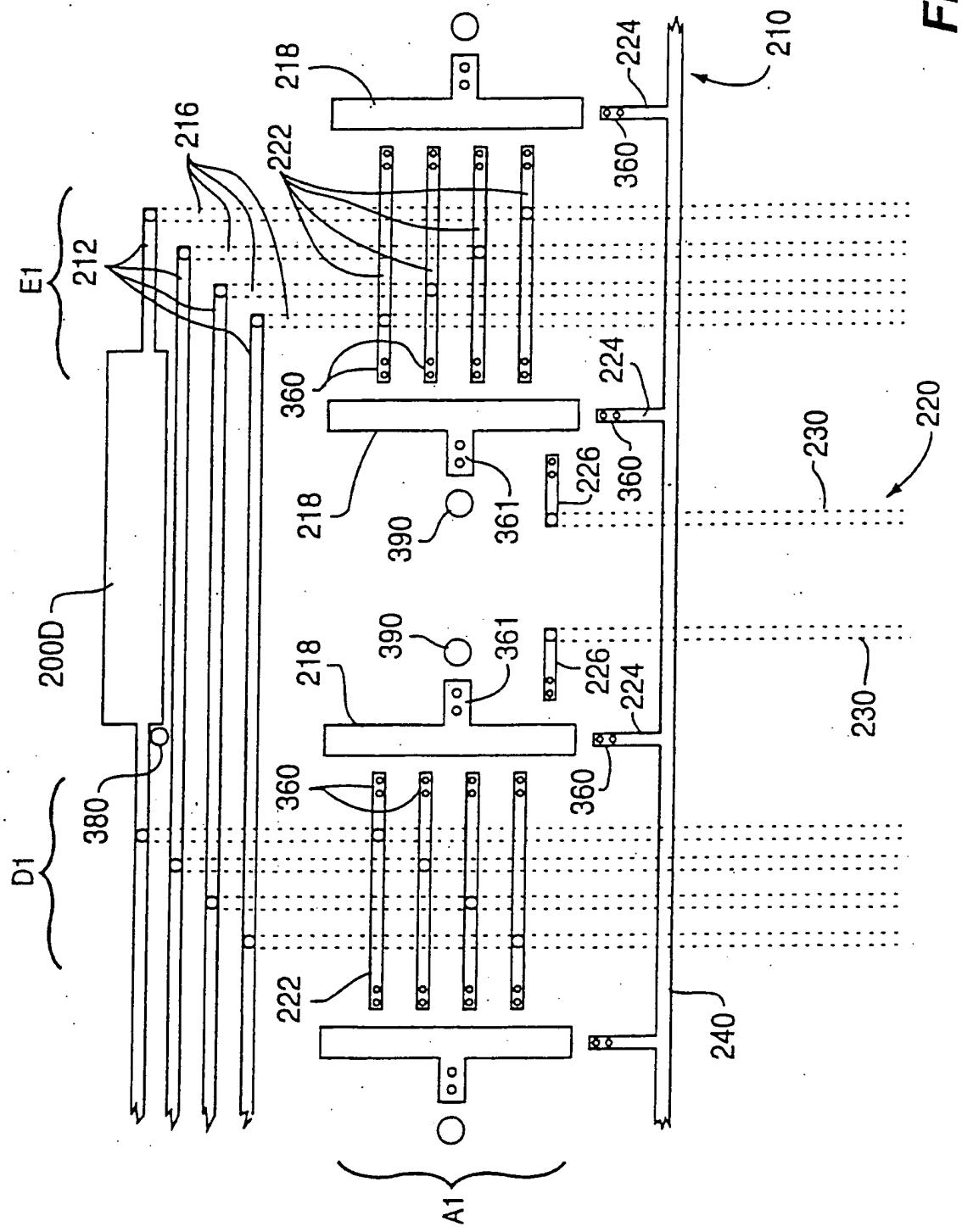
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FIG. 2

FIG. 3

FIG. 4A

FIG. 4B

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FIG. 5

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FIG. 6A

FIG. 6B

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FIG. 8

FIG. 7

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FIG. 9

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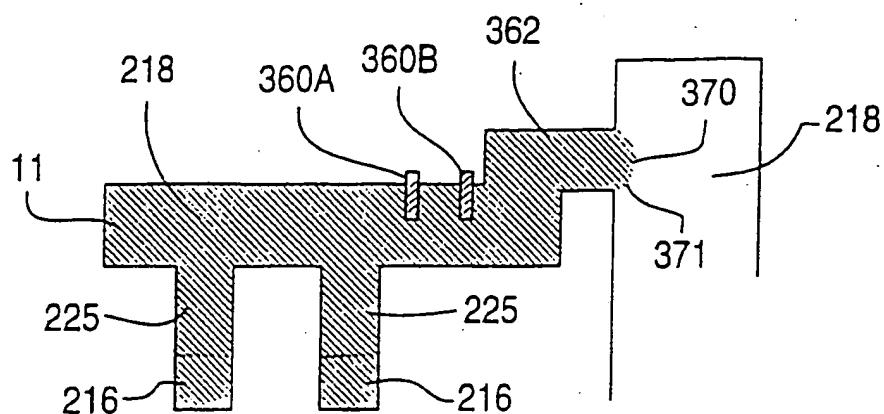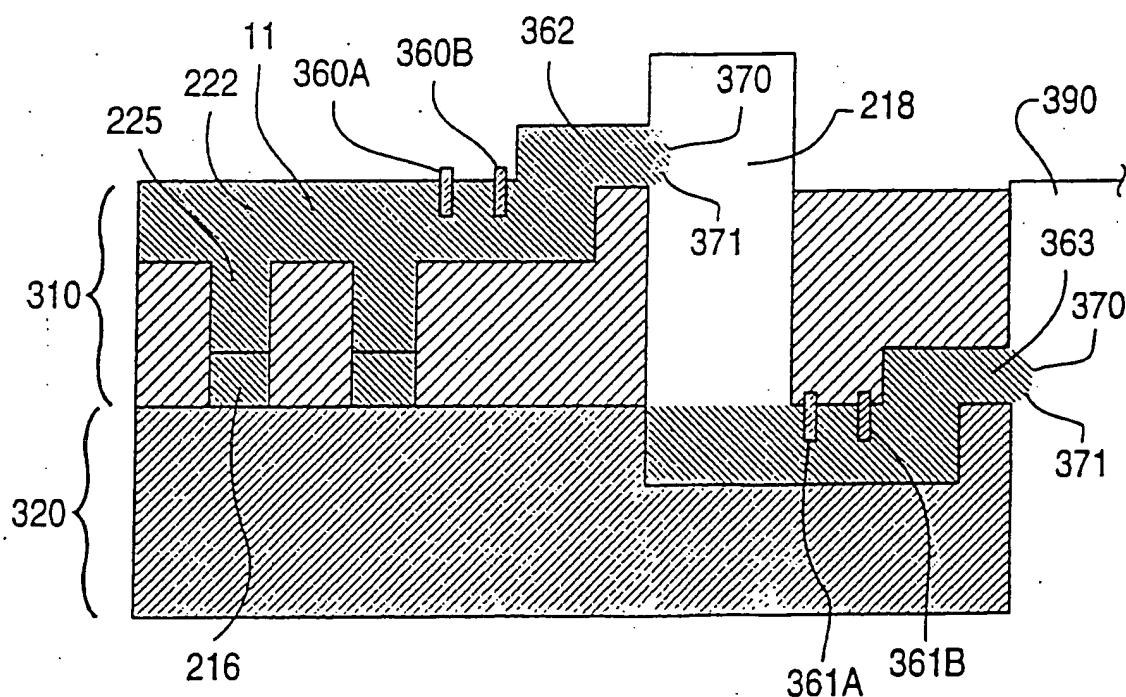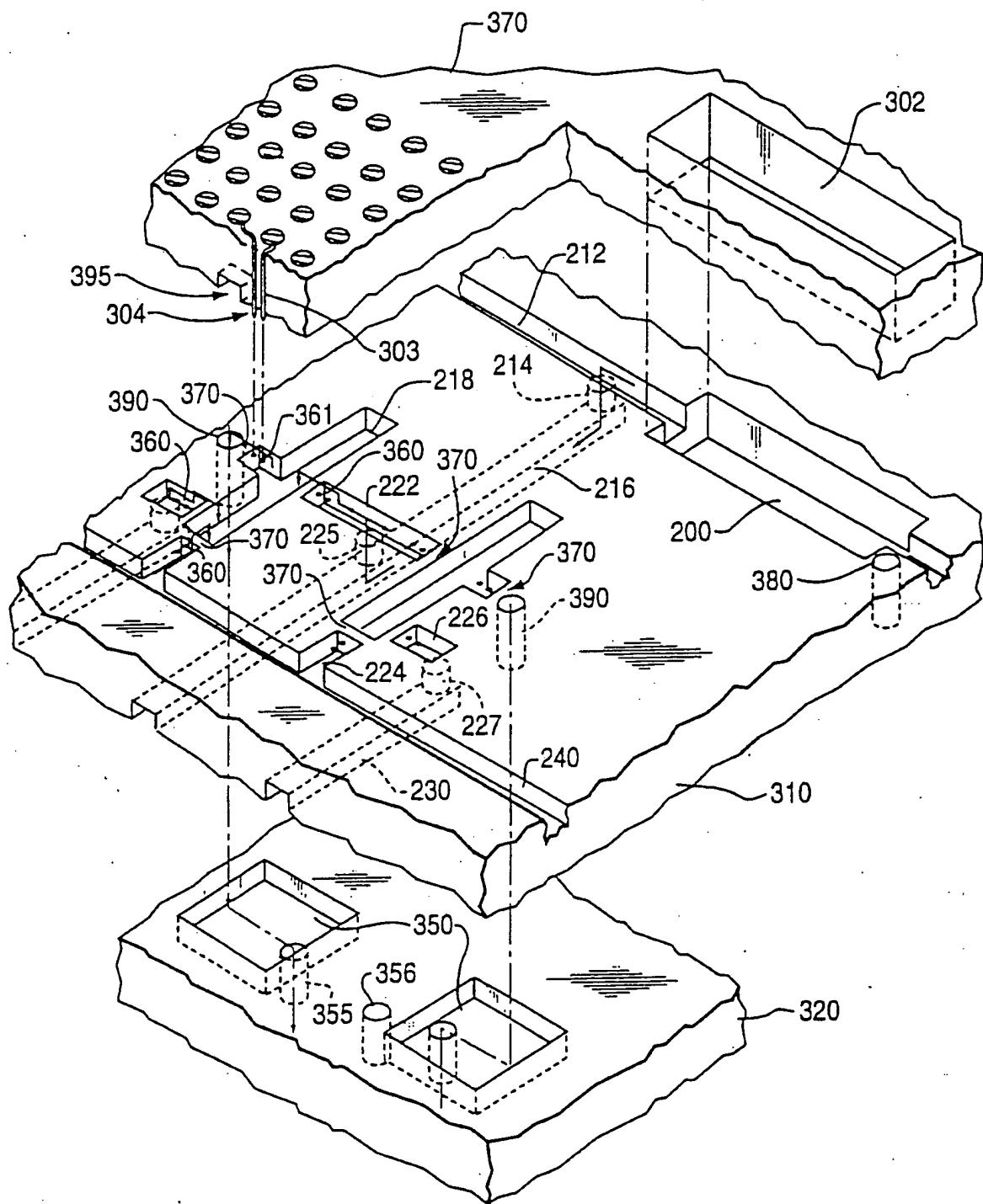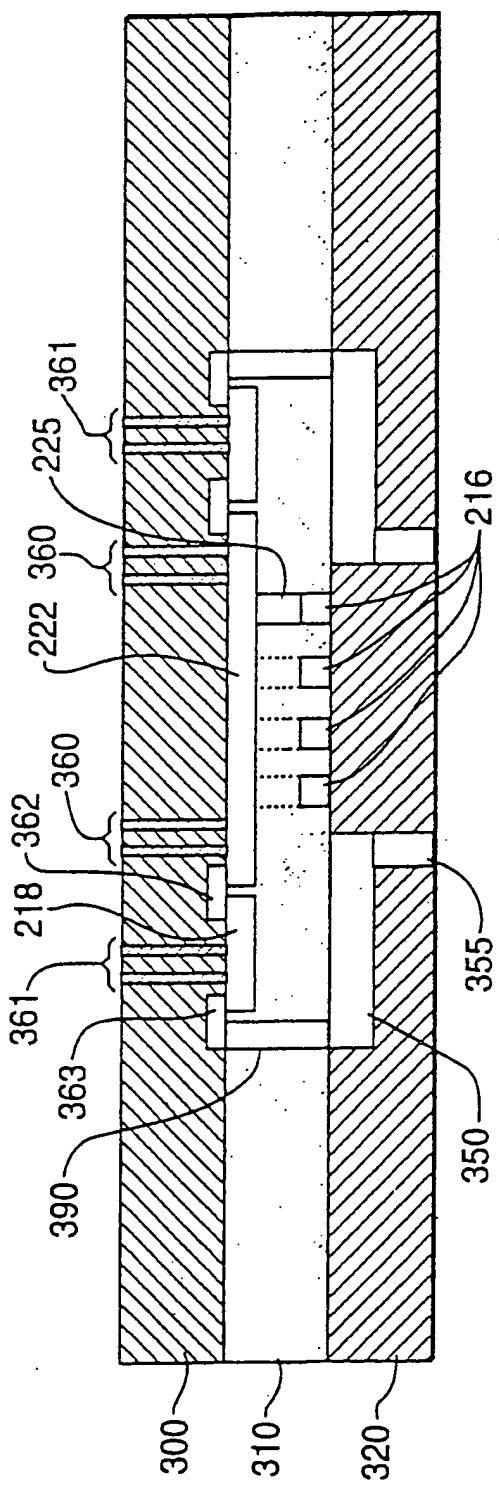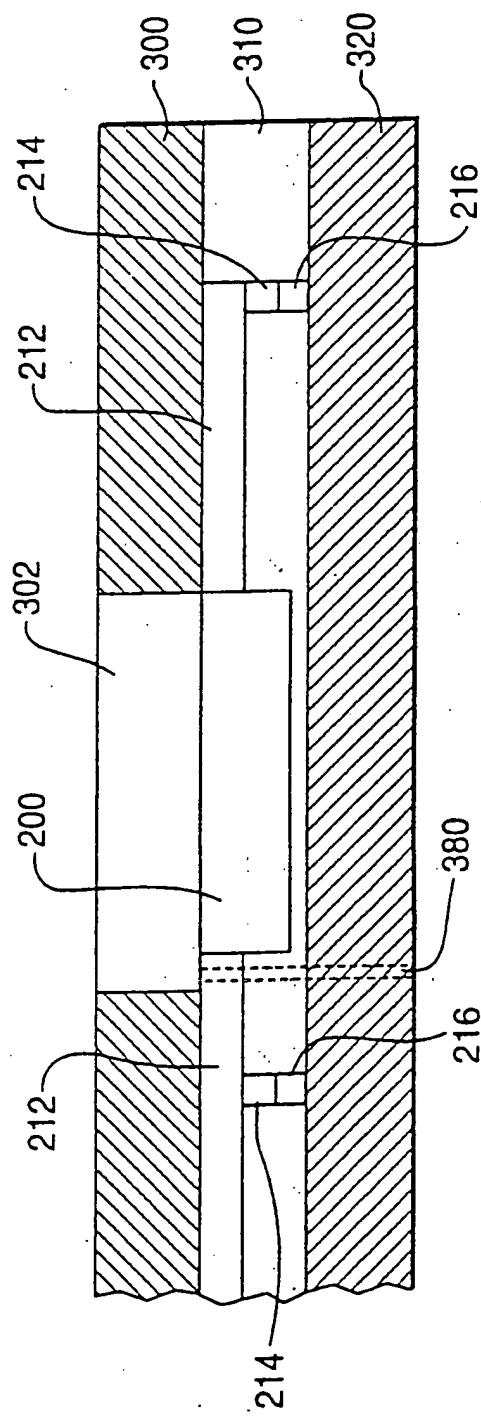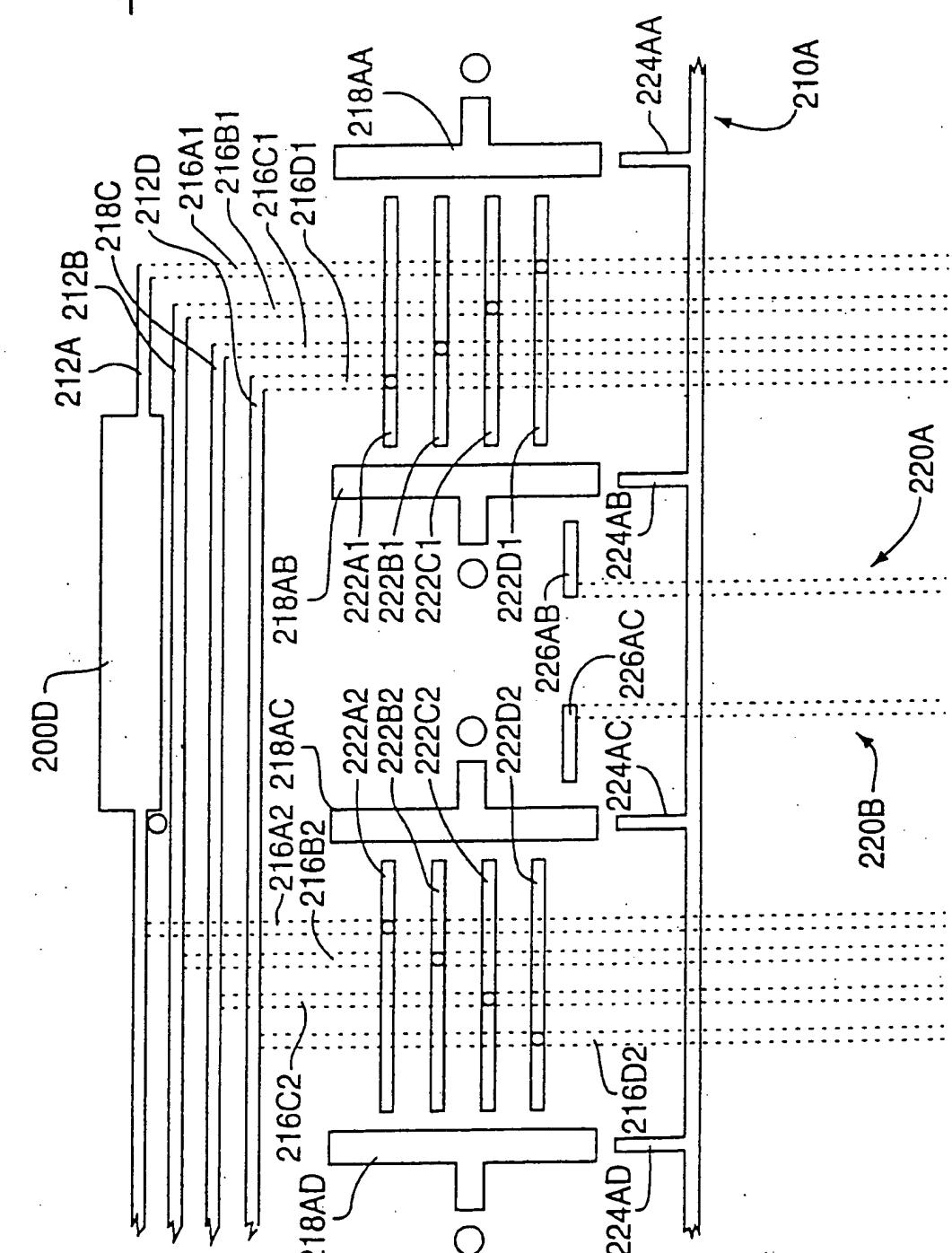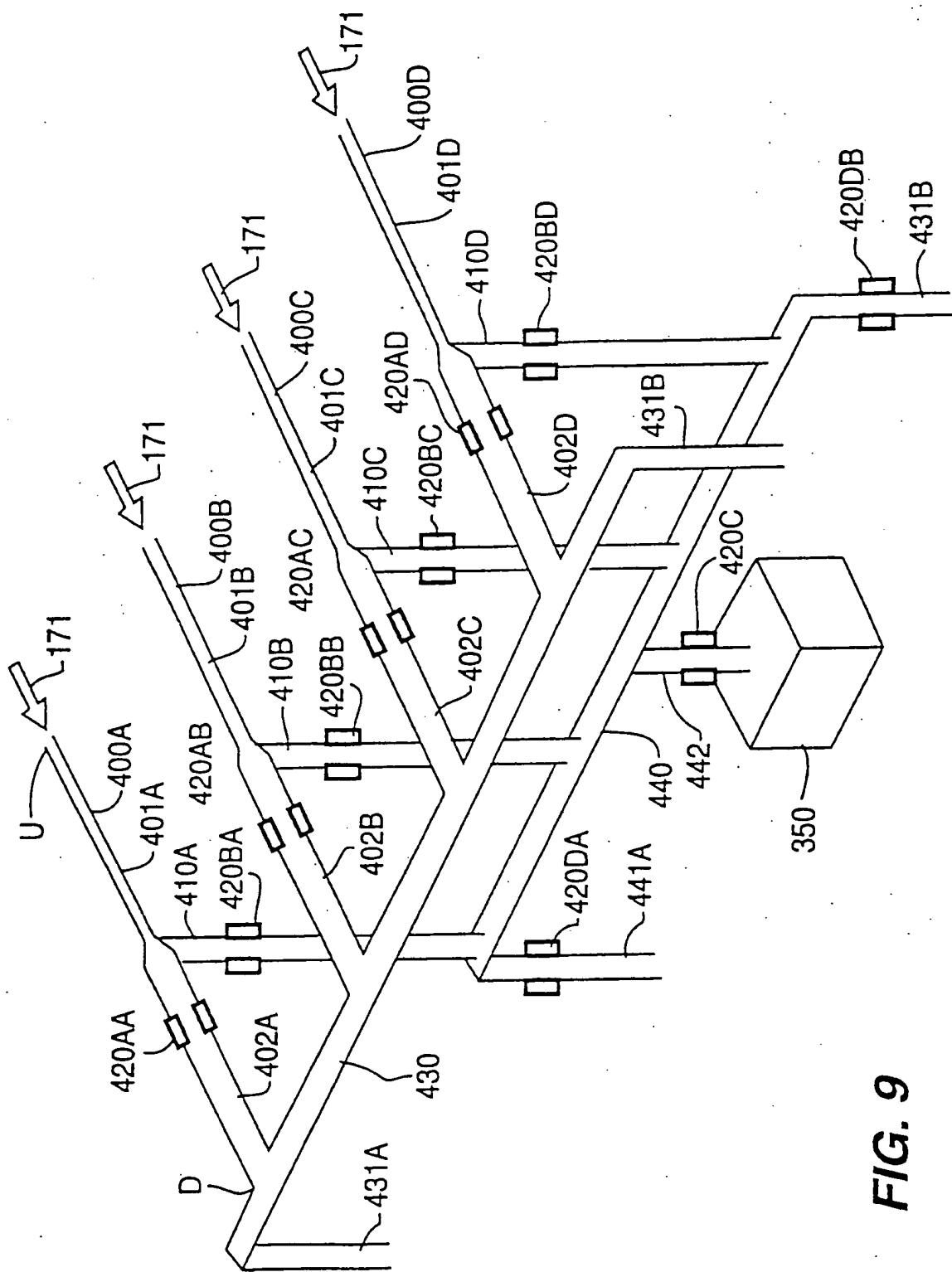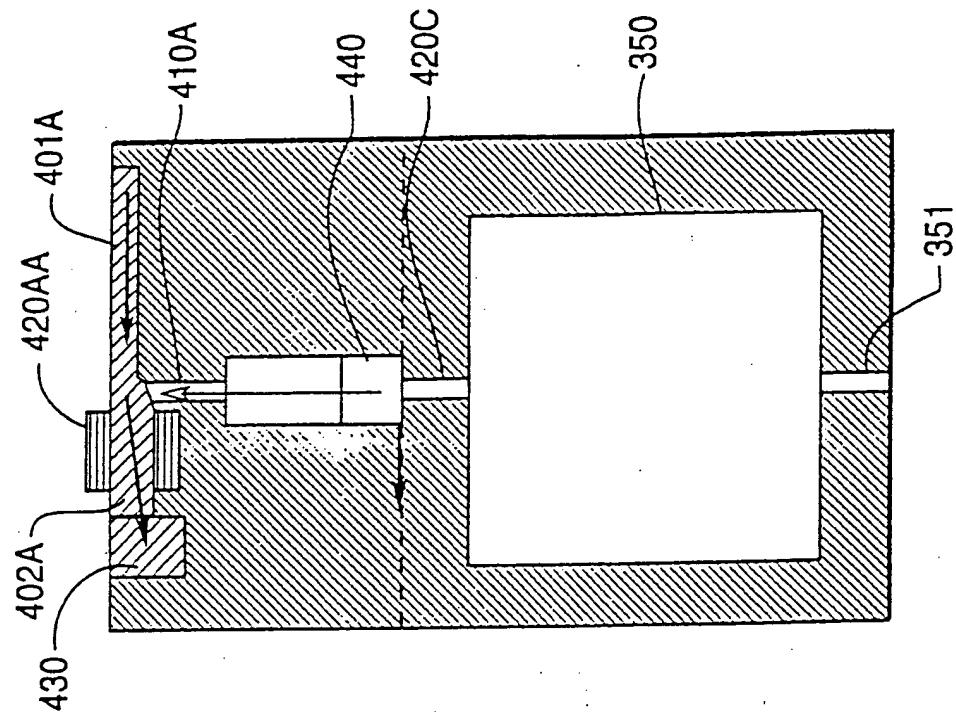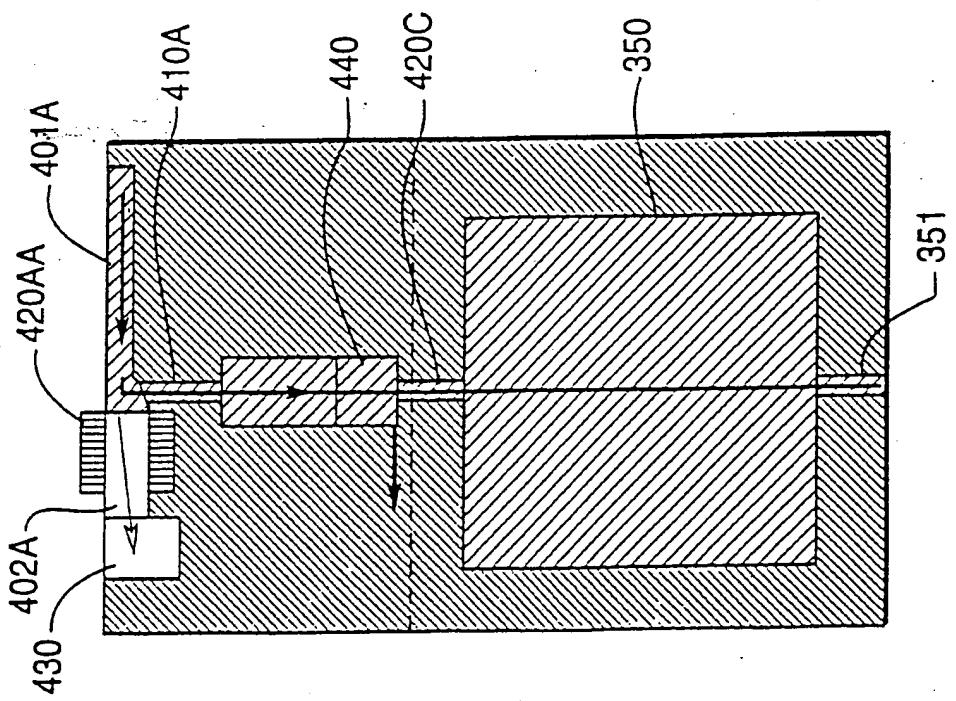
FIG. 10B

FIG. 10A

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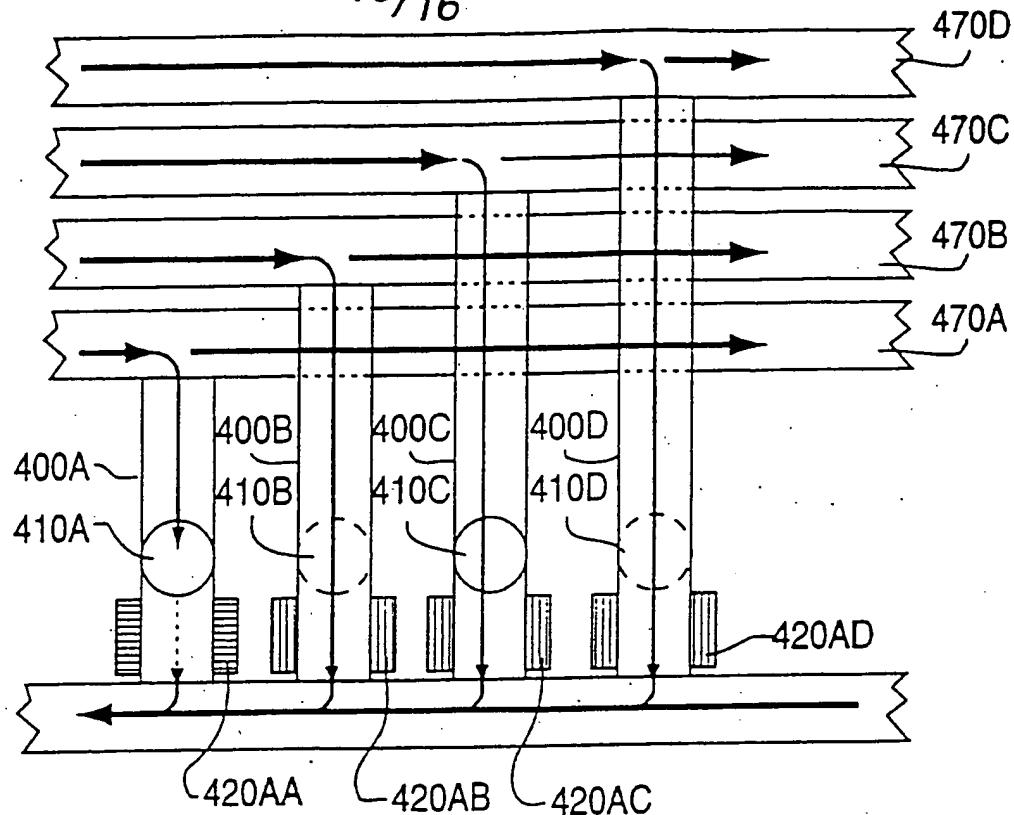
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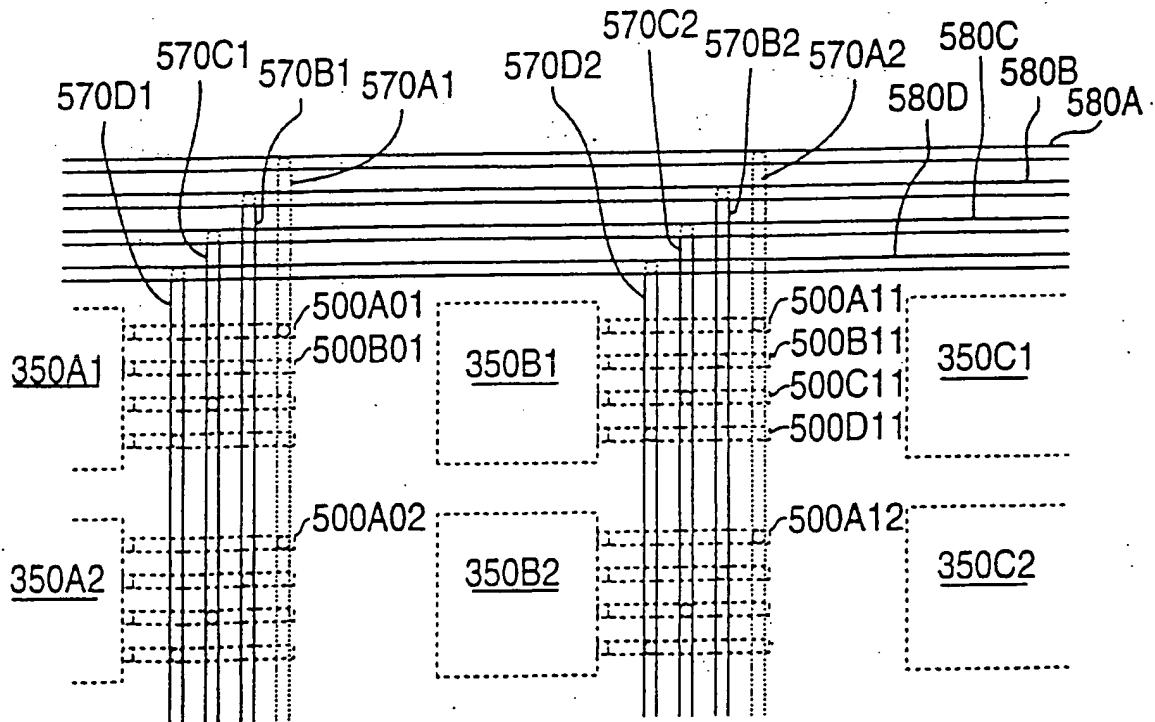
FIG. 12

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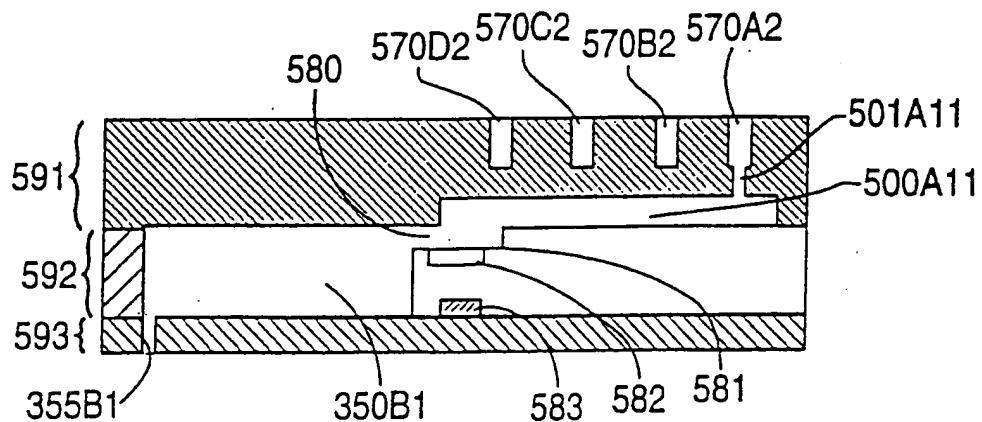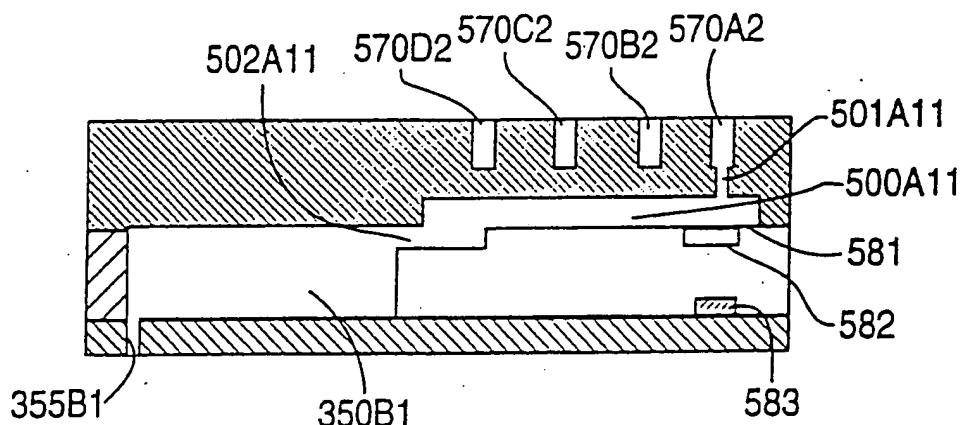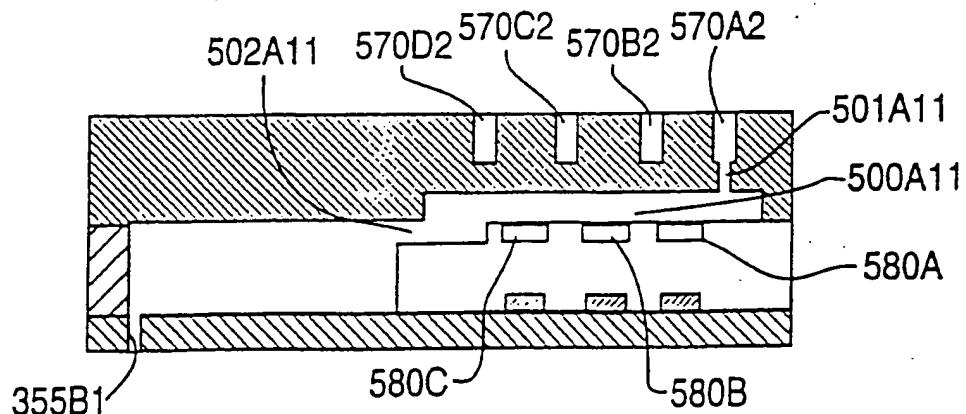
FIG. 13A

FIG. 13B

FIG. 13C
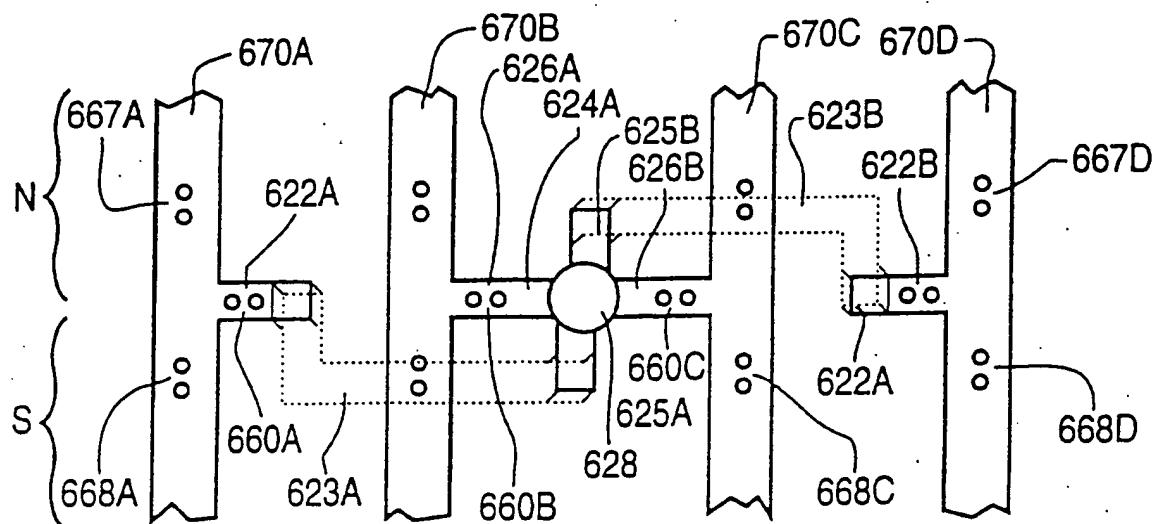
FIG. 14
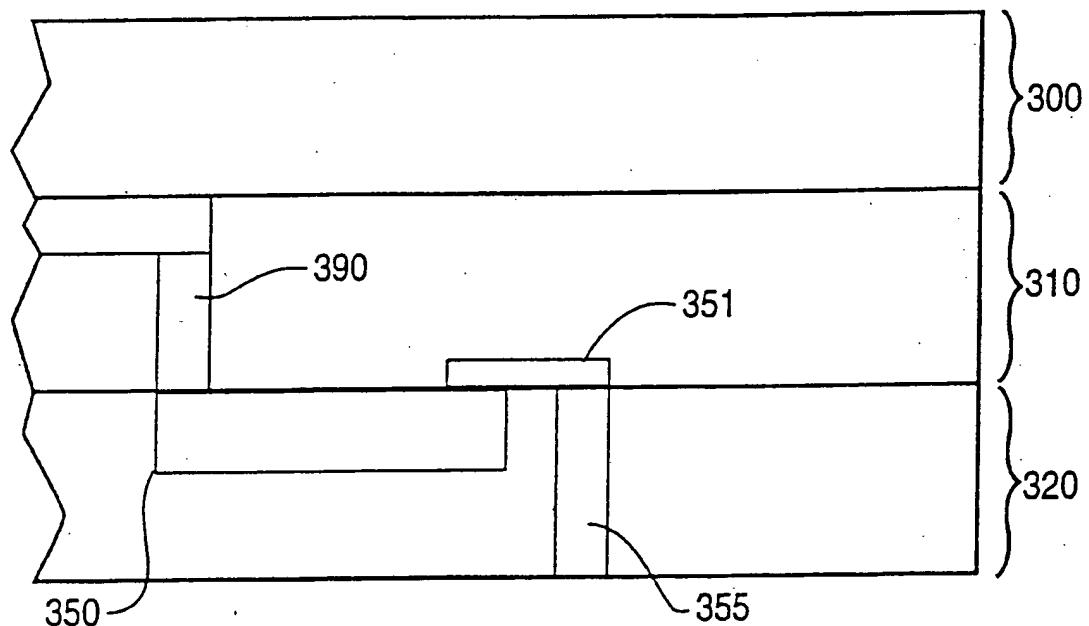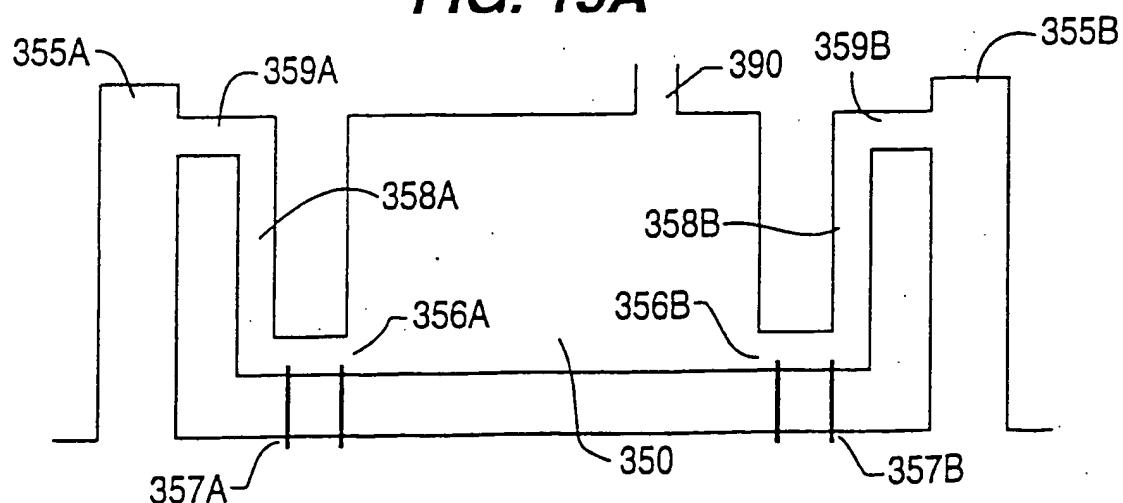
**FIG. 15A**

FIG. 15B
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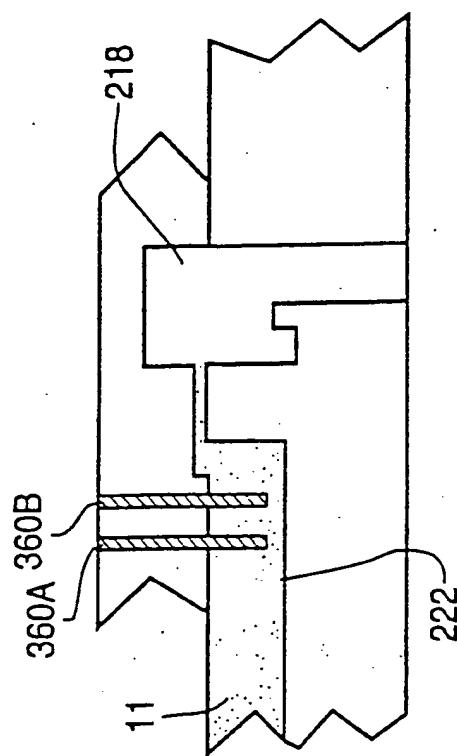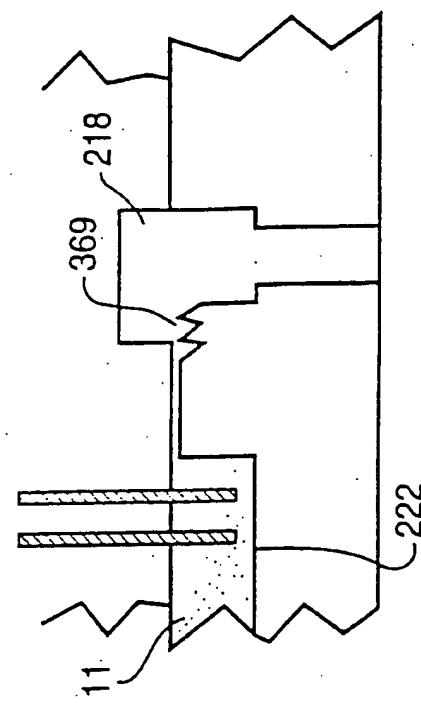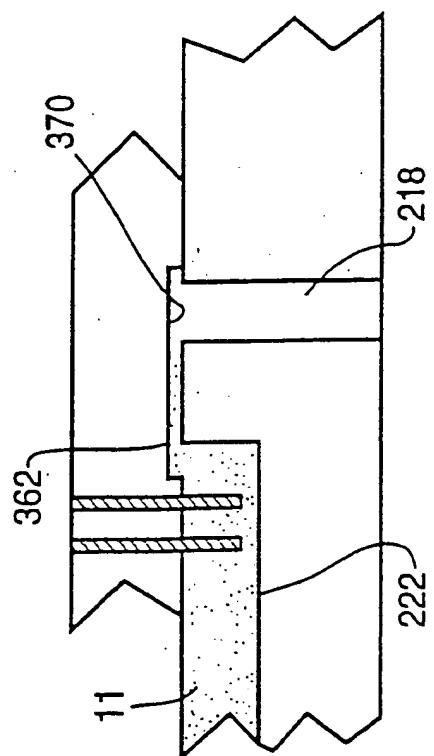
FIG. 16D

FIG. 16C

FIG. 16A
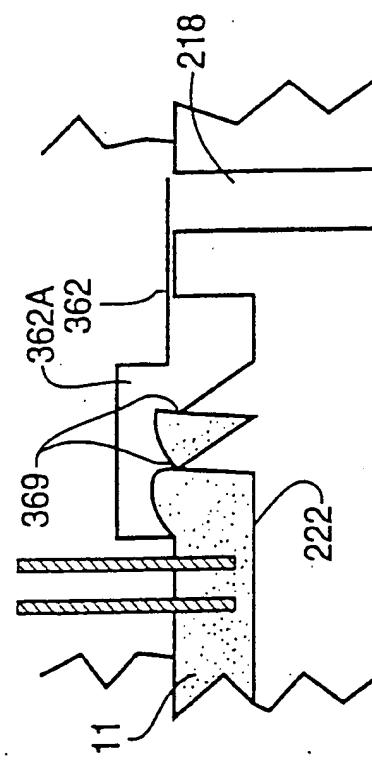
FIG. 16B

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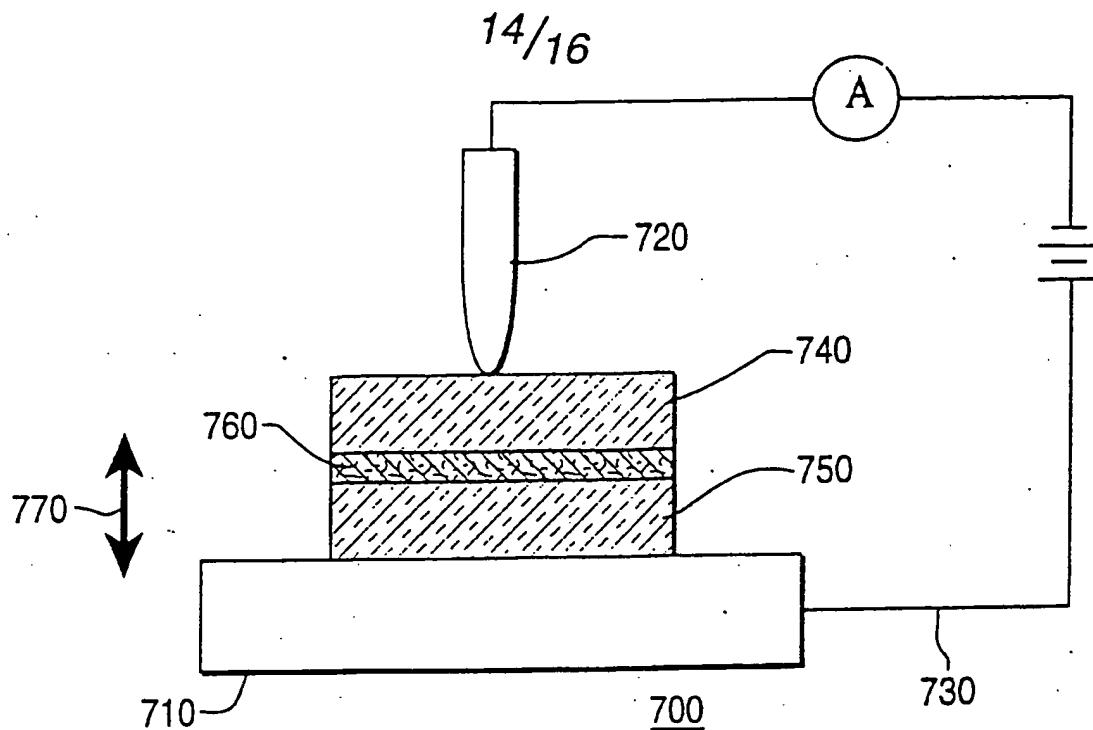
FIG. 17
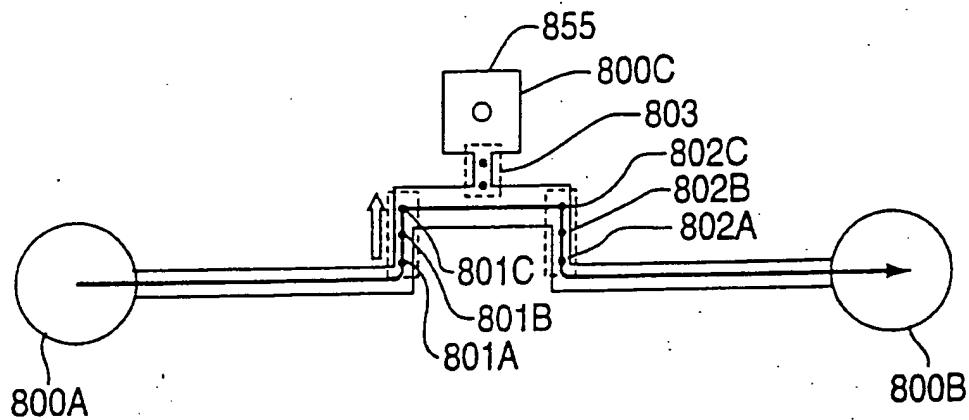

FIG. 18A

FIG. 18B
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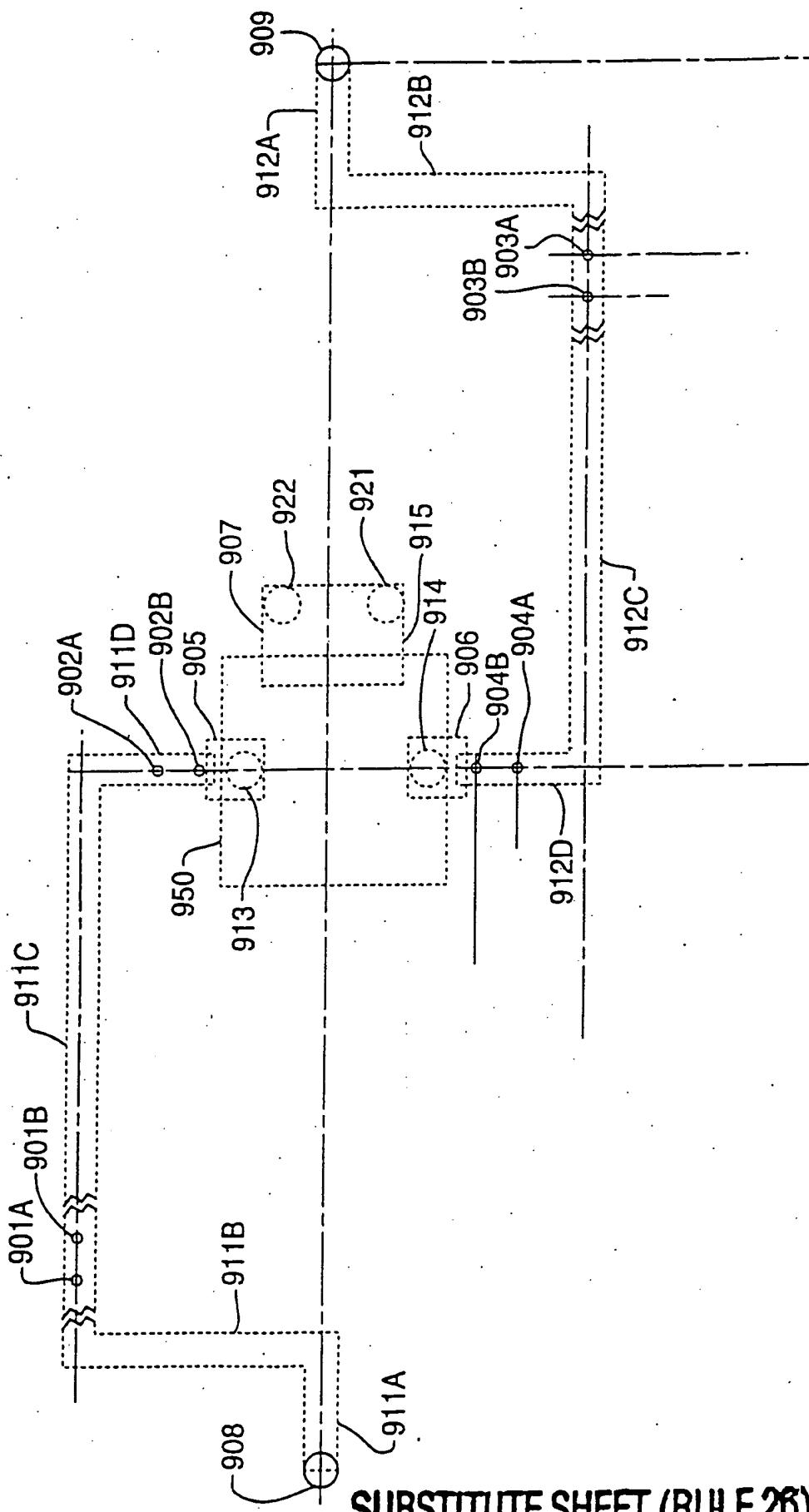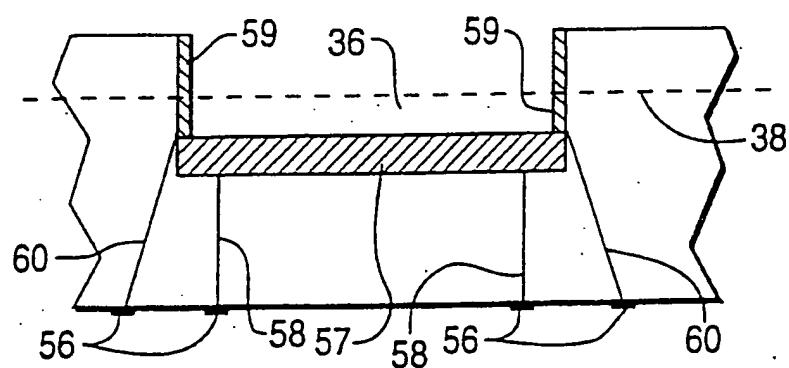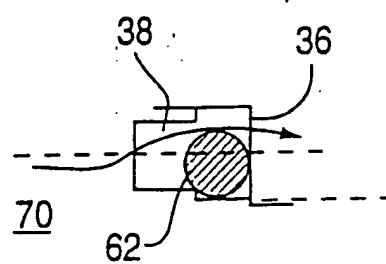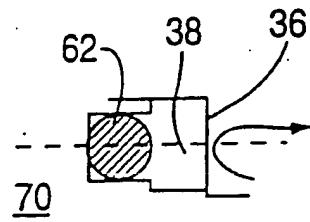
FIG. 19

FIG. 20

FIG. 21A   **FIG. 21B**

INTERNATIONAL SEARCH REPORT

A.	CLASSIFICATION OF SUBJECT MATTER

IPC(6) :H02K 44/02, 44/04; H01J 41/18; F04D 33/00
US CL :204/687; 417/48,50,53

According to International Patent Classification (IPC) or to both national classification and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 204/687; 417/48,50,53

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

C.	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 5,180,288 (RICHTER ET AL.) 19 JANUARY 1993, SEE CLAIMS AND FIGURES 1-7.	1-39
A	US, A, 4,412,785 (ROMAN) 01 NOVEMBER 1983, SEE FIGURES 1-4B AND CLAIMS 1-5.	1-39

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

* Special categories of cited documents:

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"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 FEBRUARY 1996	2 5 MAR 1996

Name and mailing address of the ISA/US	Authorized officer
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